Untersuchungen zur Prävalenz in Zwischen- und Endwirt, zur Bedeutung und zur Bekämpfung der Bovinen Fasciolose in der Schweiz

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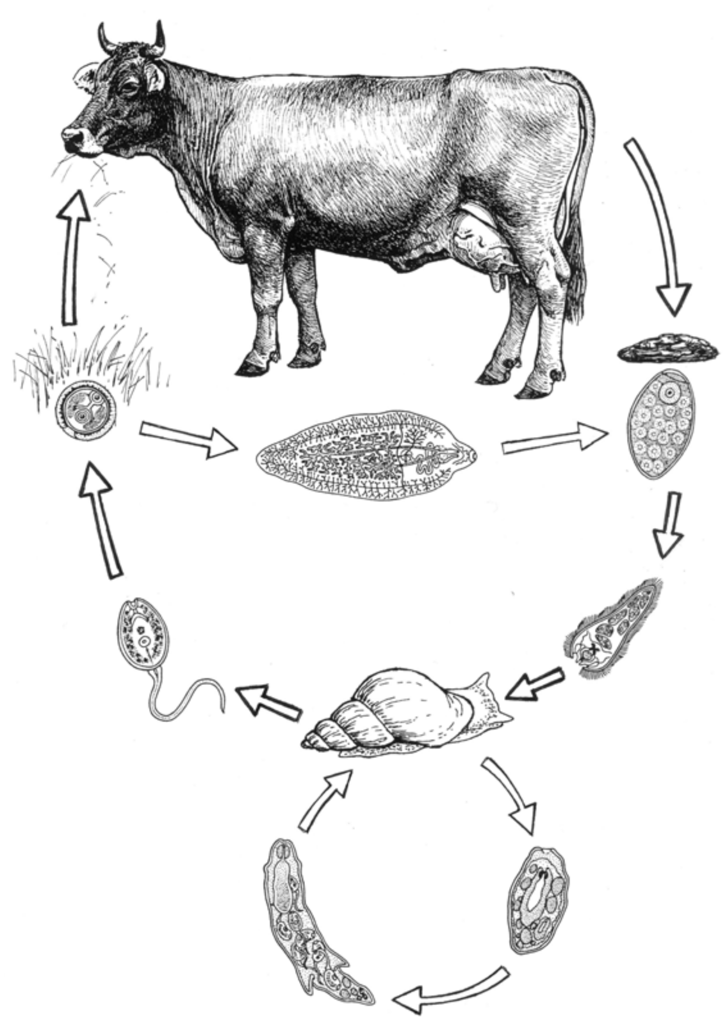
UNTERSUCHUNGEN ZUR PRÄVALENZ IN ZWISCHEN- UND ENDWIRT, ZUR BEDEUTUNG UND ZUR BEKÄMPFUNG DER BOVINEN FASCIOLOSE IN DER SCHWEIZ



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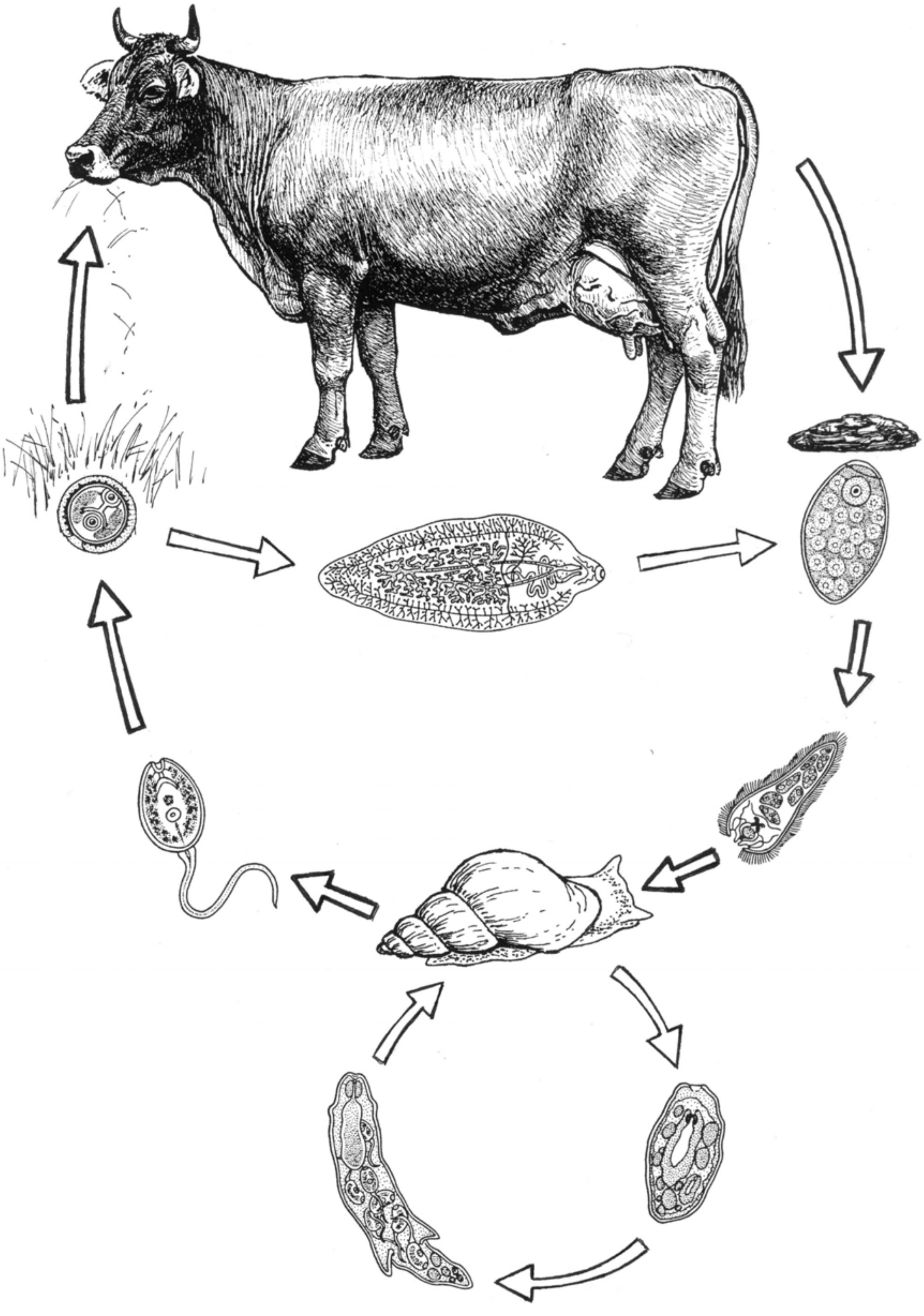

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Einleitung und Zielsetzung

Obwohl der grosse Leberegel schon seit langer Zeit bekannt und Gegenstand intensiver Forschung ist, ist das Wissen um die Bedeutung und die Verbreitung der bovinen Fasciolose in der Schweiz immer noch lückenhaft.

Informationen zur Häufigkeit und zur Verbreitung des *Fasciola hepatica*-Befalls wurden bisher hauptsächlich anhand von Schlachtvieh- oder von Kotuntersuchungen gewonnen. Die Fleischschau stellt die gängigste Methode dar, ist jedoch wenig sensitiv. Ein Ziel dieser Arbeit war die Erhebung aktueller Prävalenzdaten mit Hilfe von Fleischschau, Koproskopie und Antikörpernachweis im Blut sowie der Vergleich dieser diagnostischen Methoden, um das tatsächliche Auftreten des grossen Leberegels in der Schweiz sowie die Sensitivität und Spezifität der angewandten Untersuchungsmethoden zu ermitteln.

Der Parasitenzyklus wird über die Zwergschlammschnecke *Galba truncatula* (bisher *Lymnaea truncatula*) geschlossen. Beim Zwischenwirt handelt es sich um eine feuchtigkeitsliebende Sumpfschnecke, die überall dort vorkommen kann, wo langsam fliessende Gewässer mit sumpfigen Böden oder Ufern Lebensräume bilden. Typische Habitate sind Drainagegräben, Hangwasseraustrittsstellen, Rietflächen, die Ufer von langsam fliessenden Bächen und verschlammte Weidebrunnen. Eine in dieser Habilitationsschrift integrierte Studie untersucht die Prävalenz im Zwischenwirt in Abhängigkeit von der Art des Habitats mit dem Ziel, Informationen zum Infektionsrisiko für den Endwirt zu erhalten.

Die Kenntnis der Schneckenhabitate und somit der Ansteckungsquellen auf einem Betrieb spielen bei der Bekämpfung eine wichtige Rolle. Obwohl Bekämpfungsstrategien schon lange Zeit bekannt sind, finden diese erst dort Anwendung, wo sich die Landwirte des Problems auch bewusst sind. Um das Problembewusstsein betroffener Bauern zu ermitteln, wurden Betriebe mit Rinderhaltung besucht und die Betriebsleiter zum Wissen und dem Bewusstsein um den grossen Leberegel befragt.

Beschriebene Bekämpfungsstrategien basieren entweder auf regelmässiger anthelminthischer Behandlung – und damit der Reduktion des Infektionsrisikos für den Zwischenwirt – oder auf Weiderotation, um somit das Infektionsrisiko für den End- und

Zwischenwirt zu senken. Mit dem Ziel, die Effizienz einer gezielten Bekämpfung zu prüfen, wurden ausgewählten Betrieben Prophylaxevorschläge unterbreitet. Die Prävalenz im Endwirt wurde vor Beginn der Bekämpfung und vier bis fünf Jahre später untersucht. Dabei wurden die Betriebe, welche die Massnahmen befolgt hatten, mit denjenigen verglichen, die eine ungenügende bzw. keine Prophylaxe durchgeführt hatten.

Die bovine Fasciolose kann subakut, akut und chronisch auftreten. Am häufigsten tritt sie in der chronischen Form auf. Kühe mit einem chronischen Befall weisen unter anderem reduzierte Fruchtbarkeit und reduzierte Milchleistung auf. Dies wird vom Landwirt jedoch kaum wahrgenommen, sondern erst der Leberabzug bei der Schlachtung. Dieser beläuft sich in der Regel auf einen derart kleinen Betrag, dass ihm keine grosse Bedeutung beigemessen wird. Ein Ziel dieser Arbeit ist es, die wirtschaftlichen Schäden, die durch die bovine Fasciolose in der Schweiz entstehen, zu beziffern, um die Bedeutung dieser Parasitose zu demonstrieren.

Da das Vorkommen von *Fasciola hepatica* an das Auftreten des Zwischenwirts gebunden ist, wurde auf Basis von Bodeninformationen und meteorologischen Daten eine Karte erstellt, welche potentielle Risikogebiete in der Schweiz bildlich darstellt. Ziel dieser ebenfalls in dieser Habilitationsschrift integrierten Studie war es, Landwirte und Tierärzte für diese Parasitose zu sensibilisieren und eine Hilfestellung zu deren Bekämpfung zu geben.

Das grundlegende Ziel der vorliegenden Habilitationsschrift ist es, mit Hilfe umfassender Untersuchungen an Schlachttieren, in Feldstudien und anhand von mathematischen Modellen neue Erkenntnisse zur Häufigkeit, zum Auftreten, zur Bedeutung sowie zur Bekämpfung des grossen Leberegels beim Rind in der Schweiz zu erhalten.

1. UNTERSUCHUNGEN ZUR PRÄVALENZ VON *FASCIOLA HEPATICA* IM ZWISCHEN- UND ENDWIRT

1.1. Prävalenz von *Fasciola hepatica* im Zwischenwirt ermittelt durch real time quantitative PCR in Populationen von 70 Rindviehbetrieben aus der Schweiz

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Zusammenfassung

Bovine Fasciolose ist eine wirtschaftlich bedeutende Parasitose. Quantitative real-time PCR wurde eingesetzt, um die Prävalenz von *Fasciola hepatica* im Zwischenwirt *Lymnaea truncatula* auf 70 ausgesuchten Schweizer Landwirtschaftsbetrieben mit Rinderhaltung zu ermitteln, und damit Informationen über das Infektionsrisiko für den Endwirt zu erhalten. Schnecken aus 130 Habitaten (36 Bächen, 21 Brunnen, 24 Drainagegräben, 33 Hangwasseraustrittsstellen, 14 Rieten, 1 Drainageschaft und 1 Teich) welche auf 71 Milchkuhweiden, 39 Jungtierweiden, 14 Mähwiesen und 6 Galtkuhweiden gefunden wurden, wurden gesammelt. Davon waren 51 Populationen mit *F. hepatica* infiziert. 4733 Schnecken wurden untersucht, davon waren 331 infiziert (7.0 %). Pro Habitat wurden 1 bis 159 Schnecken gesammelt. In einem logistischen Modell wurde, bezogen auf den Herkunftsbetrieb, Clustering der Infektion der Schnecken festgestellt. Das Risiko einer Infektion des Zwischenwirts mit *F. hepatica* war in Schneckenpopulationen aus Weidebrunnen und von Rietflächen signifikant höher als in Populationen aus Bächen. Zudem war das Risiko einer Infektion für Schnecken von Jungtierweiden und von Galtkuhweiden signifikant geringer als für Schnecken von Milchkuhweiden. Es konnte kein saisonaler Einfluss gefunden werden.

Prevalence of *Fasciola hepatica* in the intermediate host *Lymnaea truncatula* detected by real time TaqMan PCR in populations from 70 Swiss farms with cattle husbandry

Abstract

Bovine fasciolosis is an economically important parasitic disease. Quantitative real-time PCR was utilized to determine the prevalence of *Fasciola hepatica* in the snail intermediate host *Lymnaea truncatula* from 70 selected Swiss cattle farms, and to gain information on the infection risk to the definitive host. Snails from 130 habitats (36 streams, 21 wells, 24 drainage ditches, 33 spring swamps, 14 reeds, 1 drainage shaft and 1 pond) originating from 71 dairy cow pastures, 39 pastures for young stock, 14 hay fields and 6 dry cow pastures were collected. Of these, 51 populations were found to be infected with *F. hepatica*. A total of 4733 snails were examined of which 331 were infected (7.0 %). The numbers of snails collected from different sites ranged from 1 to 159 snails. Clustering of infection in snails was found on the farm of origin with a mixed logistic model with random effects. The risk of infection of *L. truncatula* with *F. hepatica* was significantly higher in populations originating from wells and reeds compared to populations from streams. In addition the risk of snail infection was significantly lower in populations collected in young stock and dry cow pastures compared to dairy cow pastures. No seasonal influence was detected.

Introduction

In Switzerland, *F. hepatica* is common in cattle with prevalence rates of 8.4 to 21.4 % (Eckert et al., 1975; Ducommun and Pfister, 1991; Schweizer et al., 2003; Rapsch, 2005; Rapsch et al., 2006). Economic losses due to bovine fasciolosis in Switzerland have been estimated at more than EUR 50'000'000 per year (Schweizer et al., 2005).

Lymnaea truncatula is the principal intermediate host of *F. hepatica* in Switzerland. It is an amphibious snail, occupying moist ground, especially smooth and firm clay grounds. Preferred habitats are shallow water, ditches and banks of slow-going streams (Frömming, 1956). Also cattle watering tanks can provide suitable living conditions (Petzold, 1989). The snail is resistant to drought (survives at least 4.5 months without water) and to frost (–8 °C several months).

Anthelmintic products available for use in dairy cows have a prolonged milk withdrawal time and therefore have to be targeted during the dry period. For this and other reasons systematic control of fasciolosis requires a sound knowledge of the epidemiology of the parasite. To investigate transmission from intermediate to final host a variety of methods have been applied. The use of tracer animals proved to be of value to investigate transmission to the definitive hosts, whilst the microscopic investigation of snails for larvae of *F. hepatica* is useful in the intermediate host. However, the use of tracer calves or sheep is expensive and the microscopic examination of snails has a low diagnostic sensitivity (Kaplan et al., 1997). Therefore, several studies have used polymerase chain reaction (PCR) to detect parasite DNA in the intermediate host (Heussler et al., 1993, Kaplan et al., 1995). In both studies repetitive DNA fragments highly specific for *F. hepatica* (> 99 %, Kaplan et al., 1995) were used as targets. Further advantages of this method are a very high sensitivity (100 %, Kaplan et al., 1995) including the detection of invading miracidium (Heussler et al., 1993, Kaplan et al., 1995).

In this study real time PCR was utilized to determine the prevalence of *F. hepatica* in the intermediate host *L. truncatula* from 70 selected Swiss cattle farms, and to gain information on the risk of infection to the definite host.

Materials and methods

Sample identification and collection in the field

Between February 1999 and February 2000, premises infected by *F. hepatica* were identified by back tracing animals that had livers condemned due to liver fluke at abattoir inspection. Other farms were identified as a result of routine coproscopy or bile inspection

(Braun et al., 1995) of cattle referred to the Department of Farm Animals. A total of 70 cattle farms situated in the northeast of Switzerland were identified and visited between August 1999 and October 2002.

The population derived of 68 dairy and 2 beef enterprises and was characterized by the following parameters: The herds had a mean size of 22 ± 9 cows (range 6 – 55; for comparison: Switzerland 2002 mean 15.7 cows/owner, www.bauernverband.ch) and 14 ± 12 (range 0 – 80) young stock. Cows were kept in tie-stalls on 59 farms and in pens on 11 farms. Grazing on pastures was permitted for a minimum of 60 days from spring to autumn. This depended on weather conditions and was according to animal welfare regulations. Mean milk yield as reported by the owners was 6360 ± 782 kg/cow/year (range 3800 – 8300; n = 60; Switzerland 2002 mean 5500 kg, www.bauernverband.ch). Animals were mainly Brown Swiss, Holstein Friesian, Red Holstein x Simmental and crossbreds.

From each farm fecal samples of five cows chosen by the farmers were examined for *F. hepatica* by sedimentation technique (Eckert et al., 2005).

Possible habitats of *L. truncatula* were identified. Habitats of interest were primary habitats with environmental conditions that allow the snails to survive through the year as described by Mehl (1932). Habitats were considered geographically independent, when they were not obviously connected or fed by the same water source.

Snails with shells larger than 4 mm were collected for a period of at least 30 minutes for each habitat and the location of each site accurately recorded. Collected snails were rinsed in tap water, identified with a magnifying glass, stored in a refrigerator for 24 to 48 hours and then frozen at –18 °C. All snails collected from each habitat were stored in a single collecting tube.

Sample preparation and PCR setup

DNA from each snail was extracted with the DNeasy 96 tissue kit (Qiagen, Hombrechtikon, Switzerland). Whole snails were individually placed in the sample tubes and the extraction was carried out according to the manufacturer's instruction. DNA was eluted with two times 150 µl instead of 200 µl of buffer AE to increase its concentration, and the samples were stored at -18 °C. For every 11th sample, one tube was provided with 50 µl of bidistilled water, serving as a negative control. To scrutinize the success of the DNA extraction, DNA concentrations of one row of each plate (8 out of 96 samples) were measured. To decrease the number of the samples for the PCR analysis 5 samples at a time were pooled and analyzed in a single run. With the method described by Leutenegger (2001) primers and probe were designed to amplify a 86 bp long target of a repetitive 449 bp-long genomic DNA fragment (clone Fhr-II, GenBank S67037; Heussler et al., 1993). DNA was amplified in an ABI Prism 7700 sequence detection system (Applied Biosystems, Rotkreuz, Switzerland) using the primers fh.243f (5'-TGC AGG ATG TCA CCG TTG TAG-3'), fh.328r (5'-AAG TAC CCA ATG CGC CTC TG-3') and the probe fh.282p (6-FAM-TGG ATC TCC TCA TCG GCT GCG A-TAMRA). Amplification reaction contained 12.5 µl TaqMan® Universal PCR Mastermix (Applied Biosystems), a final concentration of 300 nM primers (Microsynth, Balgach, Switzerland), 200 nM of fluorogenic probe (Eurogentec, Seraing, Belgium) and 5 µl of DNA in 25 µl total reaction volume. After an initial step of 2 min at 50°C required for optimal Amperase UNG activity, a denaturation of 10 min at 95°C was followed by 45 cycles of 95°C for 15 sec and 60°C for 1 min.

Eight negative and 4 positive controls (DNA isolated from adult *F. hepatica)* were included in each PCR plate.

Statistical analysis

Data was initially organised on an Excel spreadsheet and then imported into R ver 2.4.0 (The R Foundation for Statistical Computing, http://CRAN.R-project.org, source code and data file as supplementary data). A generalised mixed effect logistic model was constructed where farm of origin was analysed as the random effect. Other factors including month and year of sampling, type of habitat and habitat location were examined as fixed effects. Initially all effects were included in the model, but factors that had a P>0.2 were eliminated in a backward elimination procedure. Potential interactions were also examined and the most parsimonious model was selected with remaining significant factors. The package lner4 was used for all analysis with the family binomial used as the link function. Odds ratios were calculated from the parameter estimates and their standard errors by standard techniques.

Results

Snail habitats

L. truncatula was found in all 70 farms investigated. *F. hepatica* was found in these snails in one habitat on 40 farms, in two geographically isolated habitats (without common water supply) on 14 farms, in three habitats on 9 farms, in four habitats on 3 farms, and in five and seven habitats each on 2 farms. The total of 130 habitats was located in 71 dairy cows pastures, 39 pastures for young stock, 6 pastures for dry cows and 14 hayfields. These primary habitats were 33 spring swamps, 24 drainage ditches, 21 wells (serving as drinking troughs), 14 reeds and 36 watersides of slow moving streams. On one farm each snails were found at the waterside of a small pond and in a drainage shaft.

As the size of the spring swamps varies depending on solar radiation, rain fall and soil conditions no information on the dimensions can be given. They were all found inside the pastures. The drainage ditches and streams could be found both inside and alongside the pastures. They varied in size from two meters to the whole length of one side of the pasture. The reeds were found alongside pastures and stretched across the whole length of one side of the pasture.

In each habitat from 1 to 159 living snails were found (mean 36.4, median 22). This consisted of a mean of 60 snails in reeds, 50 in wells, 48 in spring swamps, 31 in drainage ditches and 14 in streams. A total of 4733 living specimens were collected from August 1999 to October 2002.

Evidence of the parasite cycle on the farms

The parasite cycle was found to be completed on 55 farms: on 46 farms cows were found to shed *F. hepatica* eggs, and on 9 farms with negative coproscopy snails tested positive for liver fluke by real time PCR (table 1).

Table 1: Evidence of *Fasciola hepatica* life cycle on 70 cattle farms based on coproscopy and TaqMan PCR in *Lymnaea truncatula*

		F. hepatica in *L. truncatula* (TaqMan PCR)		Total
		Negative	Positive	
Coproscopy	Positive	19 farms	27 farms	46 farms
	Negative	15 farms	9 farms	24 farms
Total		34 farms	36 farms	70 farms

Prevalence of *Fasciola hepatica* in *Lymnaea truncatula*

The global prevalence of the infection in 130 populations with a size from 1 to 159 snails varied from 0 to 100 %, with a mean prevalence of 7.0 %. Of 130 populations 79 (60.8 %) were not infected.

Table 2: Results of the mixed logistic regression model of suspected risk factors for *Lymnaea truncatula* being infected with *Fasciola hepatica*

Fixed effects	**Parameter estimate (SE)**	**Odds ratio (95 % confidence intervals)**	**P**
Intercepts	-4.59 (0.56)		
Kind of habitat			
Stream			Reference
Spring swamp	1.11 (0.59)	3.04 (0.96-9.66)	0.055
Drainage ditch	0.67 (0.57)	1.94 (0.64-5.97)	0.231
Well	1.75 (0.57)	5.77(1.88-17.65)	0.002
Reed	3.33 (0.61)	27.86(8.37-92.68)	0.000
Location of habitat			
Dairy cow pasture			Reference
Young stock pasture	-1.85 (0.27)	0.16(0.09-0.26)	0.000
Dry cow pasture	-1.51 (0.66)	0.22(0.06-0.80)	0.020
Hay field	-0.97 (0.60)	0.38 (0.12-1.22)	0.096
Random effects	Variance		
Farm	6.02	-	-

Model deviance 1813 (4727 observations), BIC 1889, AIC 1831

The mixed logistic regression model (table 2) showed, that the probability of a snail being infected with *F. hepatica* varied significantly depending on type and location of the habitat. The risk for the occurrence of *F. hepatica* in *L. truncatula* is significantly higher in populations deriving from wells and reeds than from streams, and significantly lower in populations collected in young stock pastures and dry cow pastures compared to dairy cow pastures. Clustering could be found concerning the farms. Neither month nor year had an influence on the infection risk.

Discussion

Even though the overall prevalence of 7.0 % in the intermediate host in Switzerland is similar to prevalences in other countries such as Algeria (4.0 %, Mekroud et al., 2004), France (5.1 %, Mage et al., 2002) or Spain (11.4 %, Manga-Gonzalez et al., 1991) there are clear differences of prevelances in populations from the different types and locations of habitats.

The results of the regression model demonstrated that snails from young stock pastures have a significantly lower risk of getting infected with *F. hepatica* than snails from dairy cow pastures. Young animals usually do not have patent infections when allowed to graze on pastures in spring. Over wintered metacercaria and cercaria from over wintering snails will infect the young stock in late spring on a low level but the cycle will not be completed until late summer. Before the cycle will be completed a second time, the animals are housed. This results in snail infection rates remaining at a lower level.

This is also true for snails deriving from dry cow pastures: dry cows are turned out on separate pastures for only a short time of the year, and only in small numbers. Hence, the potential infection risk for the intermediate host should be low, especially on farms, where *L. truncatula* habitats are situated only on dry cow pastures and no additional infection through lactation period will take place. As *F. hepatica* can survive up to 26 months in cattle (Ross, 1968), cows with infections from the previous year will still shed eggs when being on the infectious pasture a year later and snails are infected continuously through summer but at a low level. Furthermore, dry cows are more likely to get treated with anthelminthics than lactating cows.

A higher risk on infection was found in snails from dairy cow pastures (71 habitats). As dairy cows are on pasture for at least 60 days per year, there is a constant shed of eggs on pastures through the summer by a fairly large group of animals. In addition, lactating cows are not treated with anthelminthics.

On hay fields the snails can only be infected by deploying liquid manure. As there is no concentration of eggs in liquid manure but rather a random spread on the pasture, infection of snails by hatching miracidia should also be on a low level as the results of this study demonstrate.

In reeds and wells the risk for snails to get infected with *F. hepatica* is significantly higher than in streams. Reeds are typical *L. truncatula* habitats. Silted wells also make an ideal habitat for a stable population of *L. truncatula*. Snail populations in reeds and wells do not undergo as much fluctuation, as populations in streams. Therefore, the infection risk for each snail increases in time. Furthermore, miracidia will find snails easier in stagnant than in flowing water. From this it can be concluded that the risk of infection for cattle grazing near to reeds or on pastures with silted wells the potential infection risk is higher than alongside of streams.

No significant difference in the rate of snail infection could be detected between different sampling months. This may be because relatively few of the snails were sampled in the spring compared to the autumn. Likewise there was no significant effect of year of sampling which may be due to insufficient variability in climatic conditions between different years to affect transmission.

Although 40 of the 70 farms only had one habitat, the remaining 30 had multiple habitats. With multiple sampling of snails from each farm, the mixed model approach is justified as the problem became one of repeated measures on the farm from different environments. The deviance, the Akaike Information Criterea (AIC) and Bayesian Information Criteria (BIC) were substantially lower by including the farm as a random effect than if it had been omitted (data not shown) hence indicating the mixed effects model to be a good model description and suggesting clustering of infection at the farm level. With 4727 data points, the deviance would have to be considerably greater than that observed with the mixed effects model to indicate a poor model fit. The clustering would be due to other factors affecting transmission for which data was not available for the analysis. This would include variable stocking density on farm, variations in the length of the grazing period as well as micro-climatic factors on individual farms.

The snail habitats of this study largely corresponded with the ones described by Mehl (1932). In 57 % of the cases (40/70) there was only one habitat per farm. From this it can be concluded, that fasciolosis in Switzerland is, in most cases, focal. In such cases control should be possible by means of a pasture rotation system as described by Boray (1971, 1972). This is especially true for habitats being located on pastures for dairy cows – as it was the case for 55 % of the habitats in this study. In the cases of habitats in pastures that are grazed by young stock (23 % of the total infected habitats), strategic treatment of the animals with triclabendazole should be recommended. In addition, to prevent importation of the parasite on non endemic farms, prophylactic anthelmintic treatment of all newly purchased livestock is recommended before they commence grazing.

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1.2. Prävalenz von *Fasciola hepatica* und *Dicrocoelium dendriticum* beim Rind: Untersuchung in einem Ostschweizer Schlachthof

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Zusammenfassung

An einem Ostschweizer Schlachthof wurde wöchentlich während eines Jahres der Befall mit grossem und kleinem Leberegel beim Rind anhand der Fleischuntersuchung festgehalten. Die so erhaltene Prävalenz wurde mit Studien aus den 70-er und 90-er Jahren verglichen. Die durchschnittliche Befallsrate mit *Dicrocoelium dendriticum* hat sich nicht verändert. Obwohl die Prävalenz von *Fasciola hepatica* scheinbar leicht zurückgegangen ist, konnte eine tatsächliche Abnahme der Erkrankung nicht nachgewiesen werden.

Prävalenz von *Fasciola hepatica* und *Dicrocoelium dendriticum* beim Rind: Untersuchung in einem Ostschweizer Schlachthof

Summary

On the basis of meat inspection in an abattoir in the eastern part of Switzerland, the incidence of liver flukes in cattle was recorded weekly during one year. The prevalence was compared with surveys from the seventies and nineties. The average incidence of *Dicrocoelium dendriticum* infection did not change. Although the prevalence of *Fasciola hepatica* apparently slightly decreased, an actual decrease of liver flukes could not be demonstrated.

Fasciolose und Dicrocoeliose sind bei Nutztieren gut untersuchte Parasitosen, die unter anderem durch den Abzug der Leber vom Schlachterlös wirtschaftliche Schäden verursachen. Die grössten Verluste bei Befall mit *Fasciola hepatica* entstehen allerdings infolge Leberschädigung, die sich zum Beispiel als Fruchtbarkeitsstörung, Stoffwechselstörung oder reduzierte Milchleistung äussert. Dieser Aspekt gewinnt gerade auch mit steigender Leistung von Milchkühen zunehmend an Wichtigkeit.

Die Fasciolose und ihre wirtschaftliche Bedeutung sind schon seit langer Zeit Gegenstand intensiver Forschung. Die Bekämpfungsstrategien beinhalten neben betriebswirtschaftlichen Massnahmen – wie Weidewechselsysteme, Auszäunen oder Drainieren betroffener Stellen, Trocknen oder Silieren der Schnitte betroffener Weiden – hauptsächlich den Einsatz von Anthelminthika. Die medikamentöse Bekämpfung hatte lange Zeit den Nachteil, dass die für Milchkühe zugelassenen Präparate Wirkstoffe enthielten, welche nur die adulten Stadien von *Fasciola hepatica* abtöteten. Inzwischen steht mit Triclabendazol ein Wirkstoff mit sehr guter Wirksamkeit auch gegen juvenile Stadien zur Verfügung.

Vor dem Hintergrund der zunehmenden wirtschaftlichen Einbussen durch Fasciolose, vor allem beim Milchvieh, und den verbesserten Bekämpfungsmöglichkeiten, stellte sich die Frage, ob ein Rückgang dieser wichtigen Parasitose in der Schweiz in den letzten 30 Jahren stattgefunden hat. Zu diesem Zweck wurden an einem Schlachthof in der Ostschweiz mit Einzug aus den Kantonen Thurgau, St. Gallen, Zürich, Schaffhausen, Appenzell Ausserrhoden, Glarus, Graubünden, Obwalden und Aargau während eines Jahres einmal wöchentlich an insgesamt 45 Schlachttagen 3267 Lebern auf das Vorkommen von *Fasciola hepatica* und *Dicrocoelium dendriticum* untersucht (Tab. 1). Die Lebern wurden nach der Fleischuntersuchungsverordnung vom 3. März 1995 mittels Adspektion, Palpation und Inzision in die Facies visceralis und in die Basis des Processus caudatus mit Ausstreifen der sichtbaren Gallengänge auf das Vorkommen der Parasiten untersucht. Befallene Lebern wurden nach der Fleischhygieneverordnung vom 1. März 1995 als ungeniessbar eingestuft und als tierische Abfälle entsorgt. Verglichen wurden unsere Ergebnisse mit Untersuchungen von Eckert et al. (1975), die an zwei Schlachthöfen (Zürich und St. Gallen) insgesamt 1496 Gallenblasen auf das Vorhandensein von Leberegeleiern untersucht hatten sowie mit Angaben von Ducommun und Pfister (1991), die ebenfalls 2033 Lebern und Gallenblasen aus verschiedenen Schlachthöfen in der ganzen Schweiz untersucht hatten.

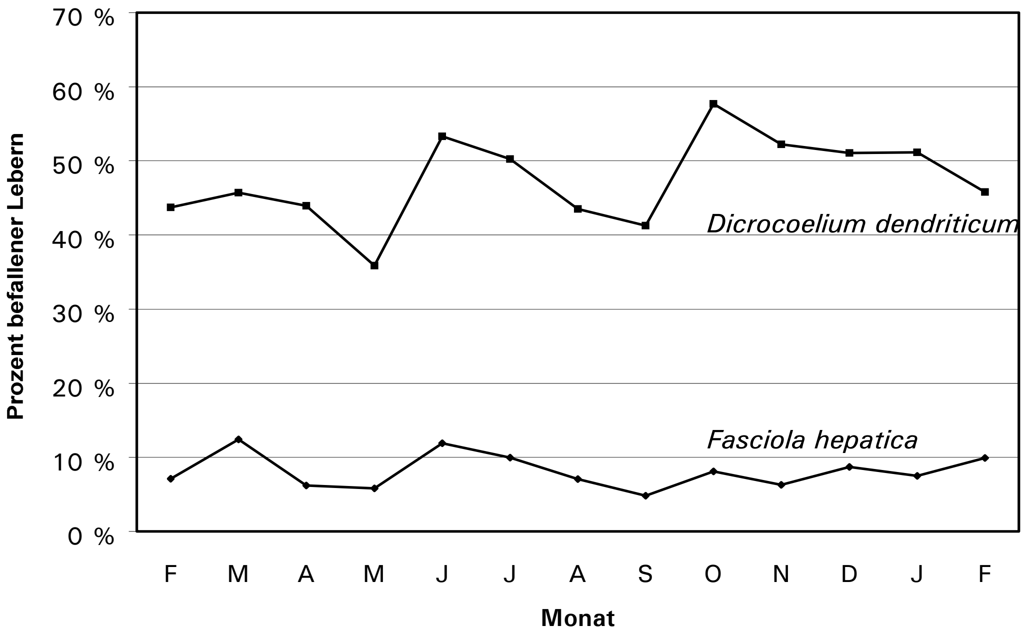
Für *Fasciola hepatica* ergab sich in den früheren Untersuchungen eine Prävalenz von 15.0 % (Eckert et al., 1975) bzw. 10.9 % (Ducommun und Pfister, 1991) und für *Dicrocoelium dendriticum* eine solche von 49.2 % (Eckert et al., 1975) bzw. 41.9 % (Ducommun und Pfister, 1991). Von den durch uns untersuchten 3267 Lebern wurden 1730 infolge Leberegelbefalls verworfen. *Fasciola hepatica* konnte bei 274 Kühen (8.4 %) und *Dicrocoelium dendriticum* bei 1543 Kühen (47.2 %) nachgewiesen werden, davon waren 87 Fälle Mischinfektionen.

Sowohl für Dicrocoeliose als auch für Fasciolose konnte zwischen den einzelnen Untersuchungsmonaten kein signifikanter Unterschied in der Prävalenz errechnet werden (Abb. 1). Während die von uns festgestellte Prävalenz von *Dicrocoelium dendriticum* sich in der gleichen Grössenordnung wie die von Eckert et al. (1975) und Ducommun und Pfister (1991) bewegte, scheint die Prävalenz von *Fasciola hepatica* in den letzten 30 Jahren leicht abgenommen zu haben, nämlich von 15.0 % (Eckert et al., 1975) auf 10.9 % (Ducommun und Pfister, 1991) und 8.4 % in der vorliegenden Untersuchung. Dabei ist allerdings zu berücksichtigen, dass die früheren Untersuchungen auf dem Nachweis von Leberegeleiern in der Galle beruhten, während sich unsere Ergebnisse auf die Leberuntersuchung stützen. Aufgrund der höheren Nachweisrate bei Untersuchungen der Galle auf Leberegeleier um ca. 13 % (Braun et al., 1995), dürfte es sich nur um einen scheinbaren Prävalenz-Rückgang handeln. Wie bei vielen anderen Krankheiten, wird es auch bei der Fasziolose schwierig sein, die Prävalenz entscheidend zu senken. Nötig ist eine noch stärkere Sensibilisierung der Tierärzte und der Landwirte für diese wichtige, die Leistung und Fruchtbarkeit mindernde Erkrankung.

Tabelle 1: Prävalenz von *Fasciola hepatica* und *Dicrocoelium dendriticum* bei Schlachtrindern an einem Ostschweizer Schlachthof

Untersuchungsmonat (1999 / 2000)	**Anzahl untersuchte Schlachttiere**	***Fasciola hepatica***	***Dicrocoelium dendriticum***
Februar	183	7.1 %	43.7 %
März	418	12.4 %	45.7 %
April	339	6.2 %	44.0 %
Mai	223	5.8 %	35.9 %
Juni	302	11.9 %	53.3 %
Juli	211	10.0 %	50.2 %
August	354	7.1 %	43.5 %
September	206	4.9 %	41.2 %
Oktober	234	8.1 %	57.7 %
November	159	6.3 %	52.2 %
Dezember	241	8.7 %	51.0 %
Januar	266	7.5 %	51.1 %
Februar	131	9.9 %	45.8 %
Total	3267	8.4 %	47.2 %

Abbildung 1: Prävalenz von *Fasciola hepatica* und *Dicrocoelium dendriticum* bei Schlachtrindern an einem Ostschweizer Schlachthof (Februar bis Dezember 1999; Januar und Februar 2000).



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1.3. Schätzung der wahren Prävalenz von *Fasciola hepatica* bei Schlachtrindern in der Schweiz in Abwesenheit eines absoluten diagnostischen Tests

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Zusammenfassung

An 1331 Schlachtrindern aus zwei Schweizer Schlachthöfen wurde eine Untersuchung zur Ermittlung der wahren Prävalenz der bovinen Fasciolose und der diagnostischen Parameter der Fleischuntersuchung, Koproskopie (Sedimentationstechnik), eines kommerziellen ELISAs zur Ermittlung spezifischer Antikörper im Serum und des Einachweises in der Galle durchgeführt. Es wurden 467 Kot-, 1269 Blut- und 995 Gallenproben entnommen. Um die diagnostische Sensitivität und Spezifität der durchgeführten Untersuchungen sowie die wahre Prävalenz der bovinen Fasciolose zu schätzen, wurde die Markov Chain Monte Carlo Methode, eine Bayesische Technik, verwendet.

Die wahre Prävalenz von *Fasciola hepatica*-Infektionen wurde auf 18.2 % (95 % Vertrauensintervall 15.0 – 20.0 %) geschätzt. Die diagnostische Sensitivität der Koproskopie wurde auf 69.6 % (50.2 – 81.4 %), diejenige der Gallenuntersuchung auf 93.7 % (85.6 – 97.3 %), diejenige des Antikörpernachweises auf 88.2 % (80.5 – 92.6 %) und diejenige der Fleischuntersuchung auf 64.0 % (53.5 – 70.8 %) berechnet. Die diagnostische Spezifität des ELISAs wurde mit 93.2 % (90.0 – 94.9 %) bewertet. Diese Resultate zeigen, dass die Prävalenz der bovinen Fasciolose höher ist, als bisher aufgrund der Fleischuntersuchung vermutet. Ausserdem konnte gezeigt werden, dass die traditionelle Koproskopie sehr effizient sein kann, wenn man wiederholt untersucht. Die Sensitivität der Koproskopie kann damit auf ca. 90 % angehoben werden.

Estimating the true prevalence of *Fasciola hepatica* in cattle slaughtered in Switzerland in the absence of an absolute diagnostic test

Abstract

A survey of 1331 cattle presented for slaughter at two abattoirs in Switzerland was used to estimate the true prevalence of *Fasciola hepatica* infection and the diagnostic parameters of visual meat inspection, coproscopy after sedimentation technique, a commercial ELISA test for specific antibody detection in serum and the post mortem microscopic detection of eggs in bile. Faeces, blood and the gall bladder were taken from most cattle presented for slaughter. In addition, livers that were rejected by the meat inspectors were also dissected to examine for the presence of liver fluke. Bayesian techniques (Markov Chain-Monte Carlo) were used to estimate the diagnostic parameters of each of these procedures and the true prevalence of bovine fasciolosis. The true prevalence of *F. hepatica* infection was estimated at 18.2 % (95 % credibile intervals 15.0-20.0 %). The diagnostic sensitivity of coproscopy, bile examination, antibody ELISA and meat inspection was estimated at 69.6 % (50.2-81.4 %), 93.7 % (85.6-97.3 %), 88.2 % (80.5-92.6 %) and 64.0 % (53.5-70.8 %) respectively. The diagnostic specificity of the ELISA test was estimated at 93.2 % (90.0-94.9 %). These results demonstrate that the prevalence of bovine fasciolosis is higher than previously thought due to the low sensitivity of meat inspection. They also demonstrate that traditional coproscopy can be very efficient if there is repeated sampling, resulting in sensitivity of approximately 90 %.

Introduction

Fasciola hepatica infects cattle and other mammalian species and is endemic in many parts of the world (Torgerson and Claxton, 1999). Previous abattoir studies have suggested that the prevalence of bovine fasciolosis in Switzerland is approximately 10 % (Ducommun and Pfister, 1991; Eckert et al., 1975; Schweizer et al., 2003). This widespread infection of the cattle population results in considerable economic losses (Schweizer et al., 2005). Crude prevalence rates are often obtained from the numbers of livers condemned which is based on the visual evaluation in abattoirs. In live animals, diagnosis has traditionally relied on faecal egg counts (Happich and Boray, 1969). More recently, serological techniques that detect circulating antibody (e.g., Cornelissen et al., 2001; Reichel, 2002) or circulating antigen (Leclipteux et al., 1998), coproantigen (Mezo et al., 2004) or eggs from bile samples taken under ultrasonic guidance (Braun et al., 1995) have been utilized. A commercial serological test with a sensitivity and specificity greater than 98 % has been reported (Molloy et al., 2005). However, this test was evaluated on two separate populations of animals and the accuracy may be subject to error, as the sensitivity and specificity of tests will vary according to the population on which they are tested (Lachs et al., 1992; Whiting et al, 2004; Leeflang and Bossuyt, 2005). Furthermore, no tests currently available can be considered as having both a 100 % sensitivity and 100 % specificity.

To overcome these problems, it is possible to perform multiple tests on populations of animals and using latent class models, maximum likelihood or Bayesian techniques to evaluate the true prevalence and diagnostic characteristics of the tests used (Enoe et al., 2000). It is feasible to estimate the true prevalence and estimate the diagnostic performance of tests if at least 3 diagnostic tests are used on the same population if no prior information is available (Toft et al., 2005). More tests may be required if the sensitivity and/or specificity of 2 or more tests are not conditionally independent. Fewer tests may be used if prior knowledge of some of the test characteristics are known.

A survey of 1331 cattle presented for slaughter at two abattoirs was used to estimate the prevalence of *F. hepatica* infection and the diagnostic parameters of four different diagnostic strategies: meat inspection, coproscopy, a commercial ELISA and examination of bile taken from gall bladders of examined livers. A Bayesian approach was utilised to calculate the unknown prevalence rate and the unknown diagnostic parameters of the test procedures.

Materials and methods

Animals

From May 2004 to August 2005, two abattoirs in the Swiss cantons of Zurich and St. Gallen were visited on several occasions. From a total of 1331 cattle presented for slaughter, faeces, blood and/or the gall bladder were taken (Table 1). Samples were taken from cattle of all age ranges (median age 4.5 years, range 4 months to 18 years). In addition, 122 livers, rejected by the meat inspectors for suspected liver fluke infection, were carefully dissected to examine for the presence of liver fluke. This was from a total of 1087 inspected visually. The remaining 965 livers were judged to be negative for liver fluke. The animals were of various breeds and included dairy and beef cattle.

Table 1: Summary of the diagnostic procedures applied to 1331 cattle at two abattoirs

Diagnostic procedure	**Number of animals**
Meat inspection, serology, bile and coproscopy	122*
Meat inspection, serology and bile	624
Meat inspection, serology and coproscopy	137*
Meat inspection, bile and coproscopy	7
Meat inspection and serology	143
Meat inspection and bile	49
Meat inspection and coproscopy	2
Meat inspection only	3
Serology, bile and coproscopy	192*
Serology and bile	0
Serology and coproscopy	15*
Serology only	36
Bile and coproscopy	1*
Bile only	0
Coproscopy only	0

*A total of 467 faecal samples were taken, of which 203 were examined on a single occasion (10 g), 8 were examined twice (2 x 10 g) and 256 were examined three times (3 x 10 g). Of the 1331 cattle, 122 were examined by all 4 diagnostic procedures, 960 by three procedures, 210 by 2 procedures and 39 by one procedure.

Samples

Faecal samples were taken and stored (for up to one month) without fixatives at 4 °C until they were analysed by coproscopy. A standard sedimentation technique was performed (Eckert et al., 2005) using 10 g rather than 6 g of faeces for the detection of *Fasciola* eggs. For many of these faecal samples the coproscopy examination was repeated 3 times in an attempt to improve the sensitivity of the test.

Gall bladders were taken and stored (for up to 2 weeks) at 4 °C until further investigation. Two 10 ml samples were then taken with a needle and syringe from the previously stirred contents, and after washing and sedimentation, examined for eggs of *Fasciola hepatica* using the same sedimentation procedure as the faecal samples.

Blood samples were centrifuged 10 min at 3500 g within a few hours of being collected, and serum was stored at -20 °C before further tested using a commercial ELISA (Institut Pourquier, Montpellier, F) according to the manufacturer's instructions.

A total of 122 condemned livers was collected and carefully dissected within two days. Major bile ducts were opened with a pair of scissors, and any *F. hepatica* was collected. Then the liver was cut into slices of about 5 cm. Smaller bile ducts were cut open and all flukes were collected. Any remaining *F. hepatica* were expressed from the tissue (Clery et al., 1996). Flukes collected were then counted.

Statistics

Bayesian techniques (Markov Chain-Monte Carlo) were used to estimate the diagnostic parameters of each of these procedures and the true prevalence of Fasciola infections in cattle. For each diagnostic test, it was assumed that the sensitivity was unknown and, hence, uniform non-informative prior distributions of between 0 and 1 were used in the analysis. Also, prior specificities of the serological and coprological tests were assumed to be unknown. Livers which had been rejected by the meat inspectors, dissected and found to be infected were assigned as infected, whilst livers not rejected or rejected but not harbouring flukes were assigned as not infected. Thus liver inspection was assigned a specificity of 1 because flukes were actually recovered from livers. The specificity of bile examination was assigned a prior specificity of 1 on the assumption that eggs detected were not misidentified, as there would be no other object in the bile that could be confused with *F. hepatica* eggs. It was also assumed that the sensitivity of egg detection in bile was correlated with the sensitivity of egg detection in faeces, as it was initially thought to be unlikely that these tests were conditionally independent (Gardner et al., 2000).

All data were entered into an Excel spreadsheet. Initial starting values of the true prevalence and unknown test parameters were assigned. From these initial starting values, the probability of each animal being truly infected based on the test results was calculated. Different numbers of tests (ranging from a single test to complete investigation of the liver, bile, faeces and serology, see Table 1) was used for different animals. However, this was always accounted for in calculating the probability of the animal actually being infected by adjusting the equation used on the relevant animal depending on the numbers of tests and the test results. For example the probability Pi of animal i being infected given the positive test results of test 1 is

$$\mathrm{P}i = \frac{\mathrm{Se}_1 \cdot \mathrm{P}}{\mathrm{Se}_1 \cdot \mathrm{P} + (1 - \mathrm{Sp}_1) \cdot (1 - \mathrm{P})}$$

where Se_1 is sensitivity of test 1, Sp_1 is the specificity of test 1 and P is the true prevalence (Smith and Slenning, 2000). Also the probability of animal *i* being infected, given a positive test result for both test 1 and test 2, is:

$$\mathrm{P}i = \frac{(\lambda + \mathrm{Se}_1 \cdot \mathrm{Se}_2) \cdot \mathrm{P}}{(\lambda + \mathrm{Se}_1 \cdot \mathrm{Se}_2 \cdot \mathrm{P}) + \left[\gamma + (1 - \mathrm{Sp}_1) \cdot (1 - \mathrm{Sp}_2)\right] \cdot (1 - \mathrm{P})}$$

where Se_1 and Se_2 represent the sensitivities of test 1 and 2, respectively, and Sp_1 and Sp_2 represent the specificities of test 1 and 2. λ is the covariance in the sensitivities of test 1 and 2 and γ is their covariances of specificities (Gardner et al., 2000). γ was always 0 as at least one of the pairs of tests with potential covariances of specificities (e.g. bile and coproscopy), was always 1. Similar equations can be derived for combinations of 3 or

4 tests and for combinations of tests for which one or more result is positive and one or more is negative.

A random binomial number of 1 or 0 was generated based on the probability of an animal being infected to give an updated disease status of each individual. From the updated disease status and test results, an updated prevalence and updated test parameters were calculated. A beta random number generator was used to update the prevalences and test parameters based on the number of infected animals and the numbers that each test correctly diagnosed as being infected or not infected. For example, the prior prevalence was a uniform (uninformative) beta distribution, with priors of both the α and β parameters of 1. If the updated disease status resulted in 200 of the 1131 animals assigned as infected and not infected, respectively, then a random number from a beta distribution with corresponding α and β parameters 201 and 1132 was generated as the updated prevalence. Thus, the updated prevalence (Updated P) varied as a beta distribution:

$$\text{Updated P} \sim \text{beta}\ (\alpha_i + \alpha_p,\ \beta_i + \beta_p),$$

where α_i and β_i are the number assigned as truly infected and non-infected (in this example 200 and 1131, respectively) and α_p and β_p are the α and β parameters of the prior distribution, in this case both are 1 as the prior is a uniform distribution.

Likewise, if the coproscopy results gave 130 of these 200 truly infected animals as positive, then an updated sensitivity of coproscopy was randomly generated from a beta distribution with α and β parameter of 131 and 71, respectively. All other updated parameters were generated in the same way. These updated values were subsequently used to generate a new set of disease states based on the new, updated predictive values and the cycle repeated. An Excel macro was written to run this Marcov chain. Random numbers were generated by an Excel add in (Excel Add-ins in Visual Basic for OR models and methods, Operation Research Group University of Texas, www.ormm.net). Each iteration was saved on to a separate row of a results spreadsheet. Once the chain had converged (or burnt in), the chain was run for a further 10,000 iterations and the median and 95 % credible intervals of the prevalence and test parameters calculated (credible intervals are the Bayesian analogue of standard confidence intervals see Basanez et al., 2004). This Marcov chain Monte Carlo assumed that there was a uniform prior distribution for all unknown parameters. However, prior knowledge of the prevalence or test characteristics could easily be incorporated into the chain. For example the model can easily be run assuming prior knowledge of test characteristics. For example, in a previous evaluation, the ELISA test was negative on 168 of 171 cattle kept in a non-endemic area of the Australian desert. The test was positive for 85 cattle of 86 with known infections (Molloy et al., 2005). These can be used as beta priors for the sensitivity and specificity of the test and can easily be incorporated into the chain as the α_p and β_p parameters replacing the uniform beta distribution where both α_p and β_p are 1 (see above).

Models were first analysed assuming covariance of test sensitivities existed. For each iteration the covariance was calculated using the formula of Gardner et al (2000) and saved to a separate line in the result spreadsheet. This assumed no prior knowledge of these parameters. When the 95 % credible intervals (CIs), calculated from 10,000 model iterations, of the covariances included 0, then the sensitivities of the tests can be assumed to be conditionally independent (Orr et al., 2003) and the parameters can be removed from the model.

The model was run under several different scenarios. This included the diagnostic result when 1, 2 or 3 faecal samples were examined from animals and when the diagnostic specificity of coproscopy was fixed at 1 on the basis that eggs were not misidentified.

Results were reported as the median and 95 % CIs, calculated from the 0.5 and 0.025-0.975 percentile respectively of the posterior distribution. The 95 % CI represents the interval in which there is a 95 % probability the true value lies. Therefore statistical comparisons of medians can easily be seen for significance if the median of one parameter is outside the 95 % CI of the comparison group. The exact p value can be calculated from the precise point on the distribution that it lies.

Results

The median true prevalence of *F. hepatica* infection ranged from 17.3 % when only one x 10 g sample of faeces was examined to 18.3 % when 3 x 10 g samples were examined and the specificity fixed at 1 (not significant). The best estimate of the prevalence, minimising the prior assumptions was 18.0 % (CIs 15.9-20.3). The diagnostic test parameters are shown in Table 2. The sensitivity of the coproscopy improved significantly from 69.0 % to 89.6 % when 3 serial samples of the same faecal sample were used rather than a single sample ($p<0.001$) (Table 2). This was mainly due to the large improvement from 69.0 % to 86.1 % by using a second sample for coproscopy. The model suggested that the specificity of coproscopy was ~ 98 % regardless of the scenario analysed suggesting a small number of eggs may have been misidentified. However, if the specificity of the coproscopy was fixed at 100 %, the sensitivity of the three serial sampling was 91.9 % and shows that the detection limit for eggs can be increased by conducting careful coproscopic analysis.

Examination of bile taken from the gall bladders confirmed that this is a highly sensitive technique for the detection of liver fluke infections, with a sensitivity of approximately 93.4 %. The liver inspection had a low sensitivity, with only 63.2 % of truly infected livers being detected by this technique. Specific antibody detection by ELISA had a “good” sensitivity and specificity of 91.7 % and 92.7 %, respectively, in the analysis using three samples of faeces with no prior assumptions regarding the specificity of coproscopy. Analysis of covariance between the faecal and bile egg detections revealed that the correlation between these tests was not significantly different from 0. Other potential correlations were analysed (e.g. between meat inspection and serology), but these were also not significant (data not shown).

Table 2: Sensitivity and specificity of the different test procedures for the diagnosis of liver bovine fasciolosis in slaughtering cattle

	Sensitivity (95 % Credible Intervals)	**Specificity (95 % Credible Intervals)**
Coproscopy x 1	69.0 % (57.3 %-79.7 %)	98.3 % (96.5 %-99.5 %)
Coproscopy x 2	86.1 % (73.2 %-94.8 %)	97.7 % (95.1 %-99.4 %)
Coproscopy x 3	89.6 % (76.4 %-96.7 %)	97.8 % (94.9 %-99.4 %)
Coproscopy x 3	91.9 % (81.3 %-97.5 %)	100 %*
Bile post mortem[1]	93.4 % (88.0 %-97.5 %)	100 %*
Liver inspection[1]	63.2 % (55.6 %-70.6 %)	100 %*
ELISA[1]	91.7 % (87.2 %-95.2 %)	93.7 % (91.7 %-0.95.2 %)

*Fixed
[1]These are based on the maximum sensitivity of coproscopy (x 3 samples) and no prior assumptions on the specificity of coproscopy. However, these values do not vary significantly between different model runs.

Discussion

The results presented give important information regarding the prevalence of *F. hepatica* in slaughtered cattle in Switzerland and the performance of diagnostic procedures for the detection of the infection. The prevalence in such animals is higher than previously thought. Earlier estimates, also based largely on abattoir surveys, have suggested that the prevalence of *F. hepatica* infections in cattle is 8.4-15 % (Eckert et al., 1975; Ducommun and Pfister, 1991; Schweizer et al., 2003). However, the present study suggests it is higher (~18 %). The difference may be due to a relatively low sensitivity of meat inspection, as the present study indicates that approximately one third of infected livers are not being detected in the abattoirs. This may have economic implications as a recent estimate of economic effects of bovine fasciolosis in Switzerland suggested that the infection resulted in losses of EURO 52 million per annum (Schweizer et al., 2005). This estimate was based on a median prevalence of 10.9 %, the true losses could be higher. Alternatively, meat inspectors may be finding the more heavily diseased livers and other infected cattle may be much less affected by the parasite.

Taking a single faecal sample of 10 g for the diagnosis of fasciolosis is not a very sensitive technique, with only 69 % of truly infected animals being detected. This finding is similar to a previous study. In Viet-Nam coproscopy in which 5 g of faeces were examined had a sensitivity of 66.7 % from cattle whose infection status had been confirmed at necropsy examination (Anderson et al., 1999). However, if three subsamples are taken, i.e. a total of 30 g of faeces, the sensitivity can be increased to as high as 90 % (or 92 % if it is assumed no eggs are misidentified). With the much higher specificity, this demonstrates that traditional coproscopy methods can have a superior test performance than other test systems. The main limitation of this approach however, is the analysis of large volumes of faeces is labour intensive. Possible sources of misidentification of eggs in the faeces include the presence of other trematodes, such as paramphistomes which have morphologically similar eggs (but of a different colour).

Previous studies (Molloy et al., 2005) have suggested that the diagnostic sensitivity and specificity of the ELISA test used in the present study is 98.2 % and 98.3 %, respectively. However, these sensitivities and specificities were calculated in two distinct populations of animals: one known to be free of the parasite and another whose infection status was confirmed by coproscopy. However, this was not an ideal approach for evaluating the test properties, as they will vary with the population on which it is used (Lachs et al., 1992; Whiting et al, 2004; Leeflang and Bossuyt, 2005). The test sensitivity and specificity in the population of cattle slaughtered in the north east of Switzerland is ~92 % (CIs 87 %-95 %) and ~94 % (CIs 92 %-95 %). Although this is a "good" test performance it is, nevertheless, significantly lower than the test properties reported previously.

Other studies have also shown that serological tests with seemingly "very good" test characteristics are less efficient when evaluated in the field. For example, a diagnostic ELISA using recombinant cathepsin L-like protease as an antigen to detect antibodies against *F. hepatica* in cattle had a sensitivity of 99.1 % and a specificity 98.5 % in experimental animals (Cornelissen et al., 2001). Unfortunately, no details of the number of flukes in these experimental animals was given. However, when this test was evaluated in cattle with naturally occurring mixed parasitic infections in areas endemic for fasciolosis, the sensitivity declined to 90.2 % and the specificity to 75.3 %.

The Bayesian techniques used in this study demonstrate how diagnostic test characteristics can be resolved for a particular population of study. Although potential correlations between different tests were included in the model, these parameters were not significant in the model. This result was not expected for coproscopy and detection of eggs in the bile as these test are looking for exactly the same evidence of infection (i.e. fluke eggs). This may be because, despite the large sample size, there were very few samples that would be predicted to be positive for coproscopy that were already negative for bile eggs based on test independence because of the very high sensitivity of the latter test. A much larger sample size would be necessary to confirm the lack of conditional independence between these and other tests. The Bayesian techniques also illustrate how prior knowledge can affect the diagnostic parameters and estimated prevalence. This can be seen when there are no prior assumptions of the specificity of coproscopy. In this scenario, the data and the prior specificities of bile examination and meat inspection give a specificity of coproscopy of 97.8 % (with 3 faecal samples). This is affected mainly by 3 samples. The first was from an animal positive for coproscopy, but negative on all three other tests. The second was from an animal positive on coproscopy but negative for bile examination and serology (no data for meat inspection) and the third was positive on coproscopy and negative on serology (no other data). The positive predictive values (i.e. the probability of these animals actually being infected) are a median of 0.019, 0.051 and 0.450 respectively for these animals. Thus, given no other information they could be false positive for coproscopy. However, when the egg counts of these three animals are examined (data not shown), two of the three had two or more eggs detected in the faeces. This is further evidence that the egg identification was accurate as repeated misidentification is unlikely, and justifies fixing the specificity at 100 %. However fixing this parameter at 100 % results in a slight upward shift in the prevalence and a downward shift in the sensitivities of the other tests. This is a major advantage of the Bayesian approach. Other rational information can be incorporated into the analysis.

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2. UNTERSUCHUNGEN ZUR BEDEUTUNG DER BOVINEN FASCIOLOSE IN DER SCHWEIZ

2.1. Die Schätzung der wirtschaftlichen Verluste durch bovine Fasciolose in der Schweiz

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Zusammenfassung

Die Infektion von Rindern mit *Fasciola hepatica* ist weit verbreitet. In der Schweiz ist die Prävalenz bei Milchkühen über 16 %. Bisherige Studien zeigen, dass sogar leichtgradige Infektionen mit dem grossen Leberegel, ohne Anzeichen von klinischen Symptomen, signifikante Leistungseinbussen bei Rindern bewirken können. Deshalb wurden die einzelnen wirtschaftlichen Einbussen, welche durch *Fasciola hepatica* entstehen und in der Literatur beschrieben sind, mittels Tabellenkalkulation aufsummiert. Da es eine beachtliche Variation der veröffentlichten Leistungseinbussen gibt, wurde diese Variabilität mittels Monte-Carlo Methode modelliert. Jeder Kostenpunkt und alle Prävalenzdaten wurden einer mathematischen Verteilung zugeordnet, die die Variabilität der experimentellen Daten und/oder den Probenumfang berücksichtigte. Es wurden insgesamt 10 000 Simulationen durchgeführt, wobei jede Grösse bei jeder Simulation zufällig durch die mathematische Verteilung variiert wurde. Das Resultat deutet darauf hin, dass sich die medianen finanziellen Verluste durch bovine Fasciolose in der Schweiz jährlich auf schätzungsweise CHF 88 414 388 (EUR 56 675 890) mit einem 95 % Vertrauensintervall von CHF 14 313 081 – 251 344 655 (EUR 9 175 052) belaufen. Pro infiziertes Tier ergibt sich daraus ein jährlicher Verlust von CHF 587 (EUR 376). Der Hauptteil der Verluste entsteht durch reduzierte Milchleistung und schlechtere Fruchtbarkeit. Verluste durch schlechtere Fleischproduktion und Leberkonfiskate machen einen geringeren Anteil am Gesamtverlust aus.

Estimating the financial losses due to bovine fasciolosis in Switzerland

Summary

Bovine infection with *Fasciola hepatica* is widespread. In Switzerland the prevalence of infection is over 16 % in dairy cattle. Previous studies have suggested that even modest infections of liver fluke, with the absence of overt clinical signs, could produce significant reductions in the performance of cattle. Therefore, the financial losses attributable to *Fasciola hepatica* were estimated using a simple spread sheet model summing the individual losses that have been suggested in the literature. Because there is a substantial variability of reported production losses attributable to *Fasciola hepatica*, Monte-Carlo sampling techniques were used to model this variability. Each cost item and each data item related to the prevalence was assigned a mathematical distribution which took account of the variability of the experimental data and/or the sample size of the reported data. A total of 10,000 simulations were undertaken, with each item randomly varied through its mathematical distribution on each simulation. The results suggest that the median financial losses due to bovine faciolosis in Switzerland is approximately CHF 88,414,388 (EUR 56,675,890[1]), with likely 95 % confidence limits ranging from CHF 14,313,081 – 251,344,655 (EUR 9,175,052 – 161,118,369), per annum, which represents a median loss of CHF 587 (EUR 376) per infected animal. The major part of the losses arises from reduced milk yield and reduced fertility. Smaller amounts are lost in meat production and by the loss of edible livers.

[1] September 2003: EUR 1.00 = CHF 1.56

Introduction

Infection with the large liver fluke *Fasciola hepatica* is a significant clinical and economic problem in production animals (Torgerson and Claxton 1999). Several experimental studies have suggested that economic losses due to reduced weight gain, decreased milk yield and lowered fertility are considerable (Ribbeck and Witzel 1979, Hope Cawdery 1984). Reductions in weight gain of 4.1 – 28 % have been described in fattening or in breeding cattle (Hope Cawdery and others 1977, Oakley and others 1979, Genicot and others 1991, Johnson 1991, Marley and others 1996). In dairy cattle, fasciolosis causes a reduced fat content of the milk and a decrease in milk yield of 3.8 – 15.2 % (Donker 1970, Hörchner and others 1970, Ross 1970, Black and Froyd 1972, Randell and Bradley 1980).

Few investigations have been undertaken on the influence of *Fasciola hepatica* on fertility in cattle, but it has been demonstrated, that cattle with fasciolosis require more inseminations to achieve conception than non-infected animals (Oakley and others 1979, Hope Cawdery 1984).

In contrast only a small numbers of studies have failed to identify production deficits. Owen (1984) was not able to detect deficits in live-weight gain and Oakley and others (1979) could not find a difference in milk quantity or quality between experimentally infected and non-infected animals. However, a close examination of the results of Oakley and others (1979) suggests that the experimental design was unlikely to demonstrate a significant difference because too few animals remained in the trial through lactation. In addition, because of the life expectancy of the fluke in cattle, and the nature of the experimental infection it is likely that in these experimental animals there were very few, if any liver fluke remaining in these animals during lactation.

Subclinical fasciolosis, in particular, is often ignored as a priority due to the lack of visible clinical symptoms. However, as experimental studies demonstrate, the true losses due to reduced weight gain, reduced milk yield, reduced fertility and the loss of livers resulting from fasciolosis can be substantial. Economic analysis of losses to the agricultural economy has a degree of uncertainty because of the variability of the losses attributed to fasciolosis in various studies. To overcome these difficulties Monte-Carlo sampling techniques to model the variability in parameters of lost production were used. In Monte-Carlo sampling, random processes are simulated using random numbers in order to decide whether or not an event takes place (Thrusfield 1995, Vose 2000). Mainly they are applied in calculations where the probability of the onset of an event is difficult or impossible to calculate by algebraic methods. Thus, the multifactorial effects of *Fasciola hepatica* on animal production suggest that such an approach to the economic analysis of bovine fasciolosis is pertinent. This technique is used to estimate the median value of a range of financial losses possible, and gives the upper and lower bounds within which the true losses to the Swiss agricultural economy are likely to lie. Such economic information is important to prioritorise disease control.

Even though the prevalence of *Fasciola hepatica* in the Swiss cattle population is around 10 % (Eckert and others 1975, Ducommun and Pfister 1991, Schweizer and others 2003), as far as the authors know, there have been no attempts to assess the financial losses due to bovine fasciolosis in Switzerland. Therefore the available theoretical estimate of the economic relevance of this important parasitic disease and the high prevalence rate justifies financial analysis of losses attributable to this disease. Such information is useful to determine the cost benefit of intervention strategies as well as a justification in investment in research into this disease.

Material and methods

The overall cost of bovine fasciolosis can be calculated by the following simple expression:

$$N * I * \left(\sum_{x=1}^{x} P_x * C_x \right) \qquad (1)$$

Where N is the population size with the annual rate of infection I, with a proportion P of infected animals with symptom x. In cattle, symptoms range from productivity losses to condemned offal at the abattoir. The total cost is calculated by multiplying the unit cost, C due to each symptom x by the specific proportion P of animals affected by symptom *x*, and then summing for all the symptoms. A national approach is adopted to represent the monetary impacts of the infection on the country as a whole.

Animal health costs

Data relating to prevalence of infection, cattle population, milk and meat production in Switzerland are presented in table 1. The prevalence of bovine fasciolosis was calculated from published data (Ducommun and Pfister 1991). The cattle population in Switzerland, the total meat and milk production, the mean cost of each day of prolonged calving interval and the value of milk and meat products were determined from data provided by the Swiss farmers association.

Reductions in weight gain of 15.9 % (Genicot and others 1991), 8.3 - 28 % (Hope Cawdery and others 1977), 4.1 – 7.5 % (Johnson 1991), 6.3 % (Marley and others 1996) and 9.2 % (Oakley and others 1979) have been described in growing fattening or breeding cattle (table 2). Based on these figures, by weighting the number of animals used in each trial, a mean deficit of 6.8 % loss of weight gain was put in for further calculations.

The effect of fasciolosis on the yield and fat content of the milk of dairy cows has also been investigated (table 3). A decrease in fat content of the milk was demonstrated by Black and Froyd (1972) in infected animals. A significant increase in milk yield and fat content of the milk after treatment was found by Hörchner and others (1970). A cattle population undergoing a three-year control program with the flukicide Bilevon R® was compared to a non-treated neighbourhood population and found a significant increase in milk yield of 50 to 154 kg and an increase in milk fat of 4 to 6 kg per cow and year. A similar investigation was carried out by Donker (1970). Different cattle populations, which took part in a two- or three-year control program with hexachlorophen, were compared to similar not treated herds. At the end of the trials, the milk yield of the treated cows was clearly higher than that of the untreated animals. Other studies have suggested that there is an increase in yield in response to flukicide treatment of 8 % (Ross 1970) and 15.2 % (Randell and Bradley 1980). Furthermore, Ribbeck and Witzel (1979) cite studies, where losses between 5 and 86 % were found.

Table 1: Productive livestock, income, treatment, and prevalence of fasciolosis in Switzerland

Category	Value	Source
Number of cattle (2001)	1,611,351	Swiss Farmers Association
Mean prevalence of *Fasciola hepatica* in cattle (%)	10.9	Ducommun and Pfister (1991)
Number of beef cattle (2001)	148,480	Swiss Farmers Association
Prevalence of *Fasciola hepatica* in beef cattle (%)	2.2	Ducommun and Pfister (1991)
Number of dairy cows (2001)	711,000	Swiss Farmers Association
Prevalence of *Fasciola hepatica* in dairy cows (%)	16.4	Ducommun and Pfister (1991)
Meat production[1] (kg/y) (2001)	54,328,000	Swiss Farmers Association
Adjusted meat production[2] (kg/y) (2001)	54,399,713	
Price for meat[3] (CHF/kg) (2001)	6.48	Calculated from data of the Swiss Farmers Association
Per annum milk yield (kg/y) (2001)	5540	Swiss Farmers Association
Adjusted per annum milk yield[2] (kg/y) (2001)	5593	
Price for milk (CHF/kg) (2001)	0.77	Swiss Farmers Association
Costs per day of prolonged calving interval (CHF)	14.95	Calculated from data of the Swiss Farmers Association
Average price per liver (CHF)	12.50	Personal communication Proviande[4]
Average price per service (CHF)	55	Personal communication SVKB[5]
Total value of Swiss agricultural production (CHF) (2001)	7.2×10^9	Swiss Farmers Association
Mean number of cattle per stock (2000)	31.2	Calculated from data of the Swiss Farmers Association
Mean number of triclabendazole applications (cows/year) (1992-1997)	12,000	Personal communication Novartis

[1] Oxen, heifers and bulls
[2] Performance adjusted for a population with 0 % *Fasciola hepatica*
[3] Mean oxen, heifers and bulls; percentage adjusted
[4] Organisation of the Swiss meat industry
[5] Swiss Association for Artificial Insemination

Based on the data by Ross (1970), Oakley and others (1979) and Randell and Bradley (1980), by weighting the number of animals used in each trial, a mean loss of 11.5 % in the milk yield of lactating dairy cattle was suggested. Oakley and others (1979) could not find a difference in milk quantity or quality between experimentally infected and non-infected animals.

Fewer investigations have been undertaken on the influence of *Fasciola hepatica* on fertility in cattle (table 4). Oakley and others (1979) describe a significant difference in conception rate in experimental heifers with fasciolosis and birth weight of the calves born to these cattle. Hope Cawdery (1984) cites Romaniuk (1977), who found that infected cows required a mean of 2.25 inseminations for conception whilst non-infected cows require only 1.62 inseminations. In addition, a single case report describes multiple abortions in a herd of dairy cows with fasciolosis (Contreras 1976).

Table 2: Percent reductions in average daily weight gain in growing cattle with fasciolosis found in published studies

Authors	**Number of animals**	**Mean daily weight gain in non-infected or treated cattle**	**Mean daily weight gain in infected cattle**	**Percent reduction in mean daily weight gain**
Genicot and others (1991)	10	1.975 kg	1.661 kg	15.9 %
Hope Cawdery and others (1977)	16 16 16			8.3 % 17 % 28 %
Johnson (1991)	70 70	2.96 lbs 3.18 lbs	2.84 lbs 2.94 lbs	4.1 % 7.5 %
Marley and others (1996)	26	3.66 lbs	3.43 lbs	6.3 %
Oakley and others (1979)	15	698 g	634 g	9.2 %

Using the data of Oakley and others (1979) a mean extension of the service period by 13 days was calculated for infected cattle. An average of 0.749 additional services was calculated from the data of Oakley and others (1979) and Hope Cawdery (1984).

Further economic deficits arise from the loss of the edible livers and the treatment of animals affected by fasciolosis. From 1992 to 1997, 12,000 cattle in Switzerland have been treated with triclabendazol (Fasinex®, Novartis) per annum at a cost of CHF 7.50 (EUR 4.81) per treatment.

Table 3: Economic effects due to reduced milk yield in dairy cows with fasciolosis

Authors	**Number of animals**	**Economic deficit**
Black and Froyd (1972)	61	Reduced fat content
Hörchner and others (1970)	not given	Significant reduction in milk yield and fat content
Donker (1970)	20 farms	Clear reduction in milk yield
Ross (1970)	32	8 %
Randell and Bradley (1980)	67	18.3 %
Oakley and others (1979)	30	0 %

Table 4: Economic effects due to reduced fertility in cows with fasciolosis

Authors	**Number of animals**	**Economic deficit**
Oakley and others (1979)	30	Extension of service period by 13 days
		0.868 additional services
		Significant reduced weight of calves at birth
Hope Cawdery (1984)	not given	0.63 additional services

Analysis

A spread sheet model was constructed using Excel (Microsoft, Redmond, WA). This consisted of summating all symptoms variables relating to animal health losses resulting from fasciolosis (equation 1). Losses due to the various symptoms and the prevalence rate of fasciolosis were estimated from the literature (table 1 – 4). The population size and the value of livestock products were taken from the homepage of the Swiss Farmers Association (www.bauernverband.ch, table 1). Losses due to each symptom, data related to prevalence, population size and values of livestock products were assigned a probability distribution. These were chosen based on the variability reported in the literature, sample sizes of experimental groups reported in the literature, and sample sizes reported for prevalence estimates. For example, the estimate of the prevalence in dairy cattle was based on a sample of 1236 livers, from dairy cattle from all parts of Switzerland, reported by Ducommun and Pfister (1991). Of these, 203 were infected with liver flukes. Hence a binomial distribution was constructed based on 1236 trials with a probability of 0.164 of each trial.

In this case

$$P\{x=i\}=\left(\frac{n!}{i!(n-i)!}\right)p^{i}(1-p)^{n-i}$$

For $i = 0,1,2,\ldots,1236$; $p = 0.164$; $n = 1236$.

This distribution was then used to model the probability distribution of the prevalence of fasciolosis in dairy cattle in Switzerland. The probability distributions of each variable and the rationale is illustrated in table 5. Essentially the methods used were similar to those described by Torgerson and others (2001) to estimate economic losses attributable to echinococcosis.

The data was entered in a Microsoft® Excel 2000 spreadsheet. The variability of prevalences, percentage decrease of weight gain and milk yield, increase in the number of days to conception, additional services, cost of a liver, number of cattle treated with triclabendazol and number of cows per stock was modelled by Crystal Ball Software (Decisioneering, Denver, CO) using Monte-Carlo sampling techniques. A single simulation calculated the total cost of the disease based on a value for each item randomly chosen from the prior assigned probability distribution. This was repeated 10,000 times in order to model the sum of the prior distributions of unit costs and a posterior probability distribution of item and total costs was calculated. The lower and upper 2.5 percentile of the 10,000 simulations was used to construct the 95 % confidence intervals.

Results

The costs of fasciolosis are presented as medians and 95 % confidence interval for individual cost items in table 6. The median total deficit was estimated to be CHF 88,414,388 (EUR 56,675,890). The largest single loss was due to a reduction in the yield of infected dairy cows which represented approximately 68 %, followed by the loss due to an extended calving to conception time which represented approximately 25 % of the total losses attributable to bovine fasciolosis in Switzerland.

The probability distribution of the losses is presented in figure 1. The median estimated losses amounts to 1 % of the total value of the Swiss agricultural production of CHF 7,225,100,000 (EUR 4,631,474,359) per annum in Switzerland.

Figure 1: Frequency distribution of estimated annual financial losses due to bovine fasciolosis in Switzerland. Each vertical bar represents approximately 1% of the displayed range.

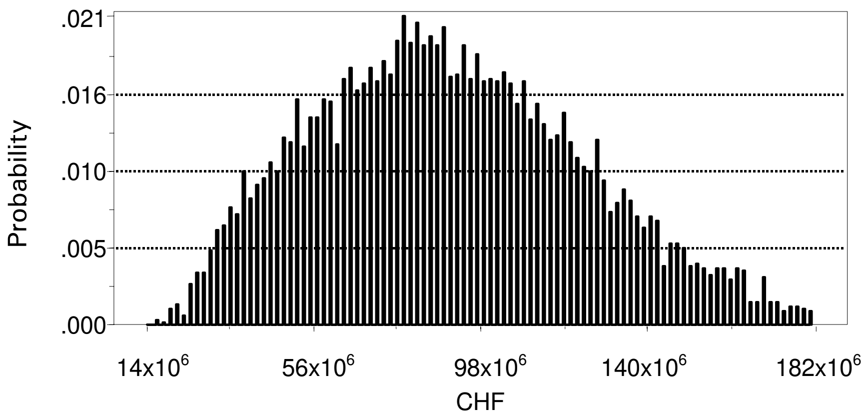

Table 5: Probability distributions of losses due to symptoms caused by bovine fasciolosis

	Distribution			Rational
Mean prevalence *Fasciola hepatica* in cattle	Binominal	1750 trials, p=0.109		Sample size of surveillance data
Prevalence *Fasciola hepatica* in dairy cattle	Binominal	1236 trials, p=0.164		
Prevalence *Fasciola hepatica* in beef cattle	Binominal	797 trials, p=0.023		
Loss milk yield	Normal	Mean 11.5 %, (+/- SD 7.6 %)	Truncated 0 minimum value	Weighted mean of values in tables 2-4
Loss meat	Normal	Mean 6.8 % (+/- SD 2.7 %)		
Extended calving to conception time	Normal	13.02 days, (+/- SD 1.3)		
Additional services	Normal	0.75 (+/- SD 0.07)		
Treated cows/year	Normal	12,000 (+/- SD 1200)		Drug sales
Economic loss per extended service day (CHF)	Normal	14.95 (+/- SD 4.5)	Truncated, 5.98 minimum, 24.21 maximum value	Calculated from data from the Swiss Farmers Association
Number of animals per herd	Normal	31.2 (+/- SD 3.1)		Official data
Price per liver	Normal	CHF 12.5 (+/- SD 1.5)	Truncated, 10 minimum, 20 maximum value	Data from Proviande

Table 6: Median estimates and 95 % confidence intervals of the per annum losses due to bovine fasciolosis in Switzerland

Category	Value in CHF	Value in EUR
Meat	519,338 (127 – 2,092,465)	332,909 (81 – 1,341,324)
Milk	59,786,853 (15,390 – 222,618,922)	38,324,905 (9,865 – 142,704,437)
Extended calving to conception time	22,391,893 (6,320,234 – 48,087,091)	14,353,778 (4,051,432 – 30,825,058)
Additional services	4,770,740 (2,575,447 – 8,358,415)	3,058,167 (1,650,927 – 5,357,958)
Loss of livers	174,424 (108,866 – 291,136)	111,810 (69,786 – 186,626)
Treatment	90,011 (53,805 – 122,348)	57,699 (34,490 – 78,428)
Total	88,414,388 (14,313,081 – 251,344,655)	56,675,890 (9,175,052 – 161,118,369)
Loss per infected animal	587 (98 – 1,853)	376 (63 – 1,188)
Average loss per dairy farm	2,958 (436 – 10,938)	1,896 (279 – 7,012)

Discussion

The adverse effect of fasciolosis on animal health and production has been investigated several times on relatively small groups of animals. Most studies suggest a significant deficit, even with a relatively modest fluke burden (Donker 1970, Hörchner and others 1970, Ross 1970, Hope Cawdery and Conway 1971, Black and Froyd 1972, Hope Cawdery and others 1977, Oakley and others 1979, Ribbeck and Witzel 1979, Randell and Bradley 1980, Genicot and others 1991, Johnson 1991). There have been few attempts to quantify the total costs of the infection on a regional or national basis. Kithuka and others (2002) estimated annual losses due to fasciolosis in Kenya to be between US$ 0.2 and 0.3 million. However, this was only based on the losses due to liver condemnations and the true economic losses are almost certainly far higher. Bennett and others (1999) used a simple spreadsheet model to estimate the annual losses due to fasciolosis in dairy and beef cattle of between £7.1 million and £50.6 million in the United

Kingdom. This previous analysis only assumed a high and low values of the losses. Deterministic methods to estimate low, medium and high losses were recommended by Bennett and other (1999) in an attempt to overcome the variability in surveillance and economic indices when estimating financial deficits. The present analysis is more sophisticated as it uses the results of various studies to construct probability distributions of production deficits to model the variability of the likely losses and gives a stochastic output of total financial losses taking into account all available information. Therefore, by using Monte-Carlo methods it has been possible not only to quantify the probable financial losses resulting from bovine fasciolosis in Switzerland, but, by including the variability suggested in previous reports, also give a reasonable accurate range within which the true loss is likely to lie. However, an obvious disadvantage of this kind of approach, and indeed more deterministic approaches, is the source of the data which is used in the model. Even though there have been many investigations on the economic importance of bovine fasciolosis, these can not always be compared among each other, since the experimental design and sometimes even the objective of the studies differ. Therefore not all the published data could be used for the calculations in this paper. Nevertheless, the methodology described in this paper models the variability implicit in the experimental data used as the source of loss estimates and is a significant advance over deterministic methods.

The largest single economic loss resulted from the losses in milk yield in lactating dairy cattle. This was followed by the costs due to an extended calving to service period. However, these two components are not always clearly separable. An extended service period also leads to a lower mean milk yield per herd (Weaver 1986). Reduced fertility in infected cattle has been investigated in controlled experimental infections. In heifers, for example, there is a delayed time to puberty and an associated increase in serum estradiol-17β and decrease in progesterone (Lopez-Diaz and others 1998) which clearly demonstrates the potential adverse effects of fasciolosis. A significant challenge in the control of fasciolosis in dairy cattle is the lack of a suitable anthelmintic which can be used in lactating dairy animals. The only available treatments have a significant milk withdrawal time and are ineffective against immature fluke. Thus, animals must be either treated in the dry period, which leads to less than ideal frequency of treatment or treatment at unsuitable times of the year to obtain optimum control. However, because losses in dairy cattle could be considerable, control of this parasitic infection should be a priority in endemic areas and alternative control methods such as pasture management to reduce the risk of exposure in lactating animals should be practiced. With a mean annual loss of nearly CHF 3000 (EUR 1896) for an average herd of 31 cattle, cost effective means of control should be achievable. The prevalence of infection in cattle is modelled from the data reported by Ducommun and Pfister (1991), which is considered representative of the whole country. A similar representative study undertaken by Eckert and others (1975), reported a prevalence of infection that was not significantly different to that reported by Ducommun and Pfister (1991), suggesting that the long term prevalence rates in cattle are reasonably stable. This is also confirmed by the most recent published data from one abattoir in the eastern part of Switzerland (Schweizer and others 2003). The magnitude of losses caused by *Fasciola hepatica* is also illustrated by the fact that in dairy farms it is similar to the total of CHF 139.44 (EUR 88.13) estimated for direct disease related costs due to a number of infectious and non infectious diseases, excluding fasciolosis (Stärk and others, 1997).

The losses due to reduced weight gain in beef cattle represent only a small part of the total deficit. This is because the beef cattle population is small compared to the total cattle population, and the prevalence of *Fasciola hepatica* in beef cattle is lower. The smallest deficits arise from the costs due to loss of the edible livers. Also a small share in the total loss arises from treatment expenses. But to date there are no investigations on calculating the true veterinary costs because of fasciolosis. It is assumed that syndromes associated with fasciolosis, such as peritonitis, dermatitis solaris or ketosis, require veterinary care. These treatment costs are likely to be higher than the ones assumed in our model. Likewise manpower costs of administration of anthelmintic treatments have not been estimated. In addition, sheep and goats are important agricultural animals in Switzerland and fasciolosis in these species could be significant. However, meat and milk prices are high in Switzerland, therefore in other countries with similar prevalence rates it is possible that financial losses would be proportionately lower.

Even though the total losses only amounts to 1 % of the total value of Swiss agricultural production (table 1), the deficits for a single farmer can be considerable. For this reason an ongoing investigation is comparing the estimated losses to the real ones.

One potential weakness of the approach described here is that cattle are assumed to be either infected or non infected. The level of productions losses may be related to the fluke burden, although this relationship is complex and non linear (Hope Cawdery 1984, Smith 1997). There is no data regarding the mean burden of fluke in affected animals. However, even modest fluke burdens are reported to result in the economic losses already reviewed. For example, a mean burden of 24 flukes per animal had a limiting effect of growth rate and food conversion (Oakley and others 1979), while a burden of 54 flukes per animal have been shown to reduce weight gain by 8 – 9 % (Ross 1970, Hope Cawdery and others 1977). Surveys suggest that naturally infected animals have considerably more than this number (Ross and others 1968).

This study reports the financial losses attributed to bovine fasciolosis in Switzerland, not the avoidable losses, or costs that can be saved as a result of appropriate intervention strategies and thus would only form part of a complete economic analysis of the disease. The total losses could only be "saved" if eradication were feasible, which is not the case with fasciolosis. Although the total losses indicate the extent of the problem, avoidable losses would give greater information with regard to the burden of disease. It has already been noted that treatment for fasciolosis in dairy cattle is limited. However, if cattle are the only livestock on the farm, intensive strategic treatments of, at most, 4 times a year would reduce the prevalence of fasciolosis to less then 1 % within a few years and less intensive treatments (e.g. once a year), following such an intervention strategy, would maintain the disease at this level (Torgerson and Claxton 1999). If sheep or goats were also present on the farm there would need to be a similar, or perhaps more intensive approach to control in these hosts as sheep are highly susceptible to liver fluke. Although recent data is lacking, reports of prevalence rates of up to 66 % in sheep in Switzerland have been documented previously (Flückiger 1964, cited in Eckert and others 1975). Therefore, the additional benefits of less disease and increased productivity in these animals would likely offset the additional costs of treatment. Reduction in prevalence rates below 1% is difficult due to wild life reservoirs such as rabbits and deer. Although triclabendazole cannot be used on dairy cattle, if an alternative were available, at a similar price, this would cost approximately CHF 927 (EUR 594) per year for a typical sized dairy herd, reducing to CHF 232 (EUR 149) within 3 – 4 years. So the initial cost of drug therapy would be less than half the average financial loss due to disease, decreasing to less then 13 %. Even if the lower 95 % cost estimate of disease on a farm was assumed, such an intervention strategy would produce a positive return. Furthermore, because fasciolosis is likely to have a clustered distribution, many farms would need little or no therapy, with a much greater return of investment on farms with higher than average prevalence rates. In contrast, the general level of infection in beef cattle is already at low levels, so would only produce an economic return if specifically targeted to high prevalence areas. Another factor that would need to be taken into account is the financial consequences of the possible development of anthelmintic resistance. There are reports of triclabendazole resistance of *Fasciola hepatica* (Moll and others 2000) and any blanket therapy to control *Fasciola hepatica* will promote the development of resistance and the financial implications of the loss of a therapeutic agent. Blanket therapy may also lessen consumer acceptance of products.

Alternative methods of control may also produce a significant return. Vaccines against *Fasciola hepatica* are being developed (Mulcahy and Dalton 2001) and if they can substantially reduce the prevalence of liver fluke in such dairy herds, they could produce a much better financial return than strategic anthelmintic treatment. However, presently the only options for dairy cattle would be anthelmintic treatment during the dry period and/or pasture management. The latter could prove cost effective if the farmer has the pasture resources so that potentially dangerous pasture can be avoided when there is a high risk of transmission. If this is not the case, then the present status quo could not be regarded as “avoidable” and an assessment if profitability and or acceptable standards of animal welfare can be achieved with the present burden of disease would be indicated.

In conclusion, this study gives a potentially useful indication of the financial effects of bovine fasciolosis in Switzerland. For a more complete economic assessment of the impact of the disease, avoidable financial losses need to be calculated and these will be dependent on control programmes designed to lower the burden of disease. These themselves will be dependent on local factors on individual farms. Studies to develop such micromodelling techniques are underway and these should give a better picture of the true economic impact of the disease.

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2.2. Eine interaktive Karte zur Ermittlung der potentiellen Verbreitung von *Lymnaea truncatula* und der freilebenden Larvenstadien von *Fasciola hepatica* in der Schweiz

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An interactive map to assess the potential spread of *Lymnaea truncatula* and the free-living stages of *Fasciola hepatica* in Switzerland
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Zusammenfassung

Der Zwischenwirt von *Fasciola hepatica* in der Schweiz ist *Lymnaea truncatula*. Sowohl die Schnecke als auch die freilebenden Parasitenstadien benötigen ein gemässigtes Klima und Feuchtigkeit für das Überleben, die Fortpflanzung und die Übertragung. Diese Bedingungen sind in der Schweiz in vielen Regionen erfüllt, weshalb die bovine Fasciolose mit einer durchschnittlichen Prävalenz von 8.4 bis 21.4 % auftritt. Eine interaktive Karte wurde entworfen um das relative Übertragungsrisiko aufzuzeigen. Dafür wurden die für das Überleben und die Fruchtbarkeit der freilebenden Parasitenstadien und des Zwischenwirts geeigneten Umweltbedingungen modelliert. Die Karte basiert auf Temperatur- und Niederschlagsdaten, Bodenbeschaffenheit inklusive Grundwasser und Forstbestand in der Schweiz. Ausführliche Informationen über Beeinflussung der Larvenstadien des grossen Leberegels und des Zwischenwirts *L. truncatula* durch diese Umweltbedingungen wurden verwendet um die interaktive Risikokarte zu erstellen.

An interactive map to assess the potential spread of *Lymnaea truncatula* and the free-living stages of *Fasciola hepatica* in Switzerland

Abstract

The intermediate host of *Fasciola hepatica* is *Lymnaea truncatula* in Switzerland. The snail and the free living stages of the parasite require a moderate climate and moisture for survival, reproduction, and transmission. In Switzerland, these conditions are present in many regions, resulting in a mean prevalence of bovine fasciolosis from 8.4 to 21.4 %. An interactive map was created in order to demonstrate the relative risk of transmission by modelling the environmental conditions that promote the survival and reproduction of the larval stages of the parasite and the parasite's intermediate host. The map is based on temperature and rainfall data, soil conditions including ground water and forest cover in Switzerland. Extensive information on the larval stages of *F. hepatica* and the intermediate host *L. truncatula* and how the development of these are affected by these environmental factors was used to create the interactive risk map.

Introduction

Fasciolosis, caused by *Fasciola hepatica*, is a serious parasitic disease in Switzerland. Transmission depends on susceptible definitive hosts (e.g. sheep and cattle) and appropriate habitats for development of both the parasite larvae and the intermediate host *Lymnaea truncatula*. This snail requires a moderate climate and moisture for survival and reproduction (Thomas, 1883). In Switzerland, these conditions are present in many regions, resulting in a mean prevalence from 8.4 to 21.4 % in cattle (Eckert et al., 1975; Ducommun and Pfister, 1991; Schweizer et al., 2003; Rapsch, 2005; Rapsch et al., 2006).

As a result of the widespread distribution of bovine fasciolosis, significant economic losses occur. This is due to confiscated livers, reduced milk yield, reduced fertility and reduced meat production. Median financial loss due to bovine fasciolosis in Switzerland is approximately EUR 52 million (95 % CI EUR 22 – 92 million) per annum, which represents a median loss of EUR 299 per infected animal (Schweizer et al., 2005). Suitable control strategies, such as pasture management strategies (Boray 1971; 1972), could help to avoid some of these losses. Geographical information systems such as risk maps could help identify areas where disease monitoring should be established. Since *F. hepatica* transmission is linked to its intermediate host *L. truncatula*, information on suitable environmental conditions can help locate possible areas with enhanced infection risk by means of cartography.

The aim of this study was to create a map based on multimedia cartography illustrating regions with good environmental conditions for the development of *L. truncatula* and the free living stages of *F. hepatica* as basis for the implementation of control strategies.

Material and methods

Risk model

The occurrence of *L. truncatula* and the transmission of *F. hepatica* mainly depend on temperature, moisture, soil conditions, and solar irradiation (Thomas, 1883; Kendall and McCullough, 1951; Ollerenshaw, 1959; Ross, 1970a; Armour, 1975; Christensen et al., 1976; Christensen et al., 1978; Petzold, 1989). Therefore, a risk model was developed based on temperature, rainfall, soil condition and forest cover as these data were readily available. The model's output is an environmental relative risk measurement for the development of *L. truncatula* and the free living stages of *F. hepatica* in Switzerland.

Environmental risk factor temperature

The occurrence of *L. truncatula* and the transmission of *F. hepatica* mainly depend on temperature, moisture, soil conditions, and solar irradiation (Thomas, 1883; Kendall and

McCullough, 1951; Ollerenshaw, 1959; Ross, 1970a; Armour, 1975; Christensen et al., 1976; Christensen et al., 1978; Petzold, 1989). Therefore, a risk model was developed based on temperature, rainfall, soil condition and forest cover as these data were readily available. The model's output is an environmental relative risk measurement for the development of *L. truncatula* and the free living stages of *F. hepatica* in Switzerland.

Environmental risk factor rainfall

It is known that snails need moisture and rainfall for survival and reproduction (Frömming, 1956).

Several forecast models are based on monthly rainfall data to predict relative monthly or seasonal infection risk (Ollerenshaw and Rowlands, 1959; Ollerenshaw and Smith, 1969; Ross, 1970b; Malone et al., 1987). But none of them is based on monthly means over several years to predict potential risk regions. Hence, the following approach was made: rainfall has a positive effect upon all free living stages of the parasite and the intermediate host. Thus, risk frequency increases with increasing rainfall, with a maximal risk at approximately 90 mm per month. However, large amounts of rainfall can wash away parasite larvae and snails and separate them from each other, thus inhibit transmission. Therefore, amounts of more than 210 mm are considered to decrease the risk frequency rapidly.

Environmental risk factor soil condition

L. truncatula prefers small water courses (pools, small streams, drainage ditches, e.g.; Frömming, 1956). It is assumed, that these small water bodies are more likely to occur with suitable soil condition and ground water, as clay soils are more water retentive than sandy soils (Ollerenshaw and Smith, 1969).

F. hepatica infection in *L. truncatula* is modelled as a function of soil conditions including ground water by an ordinal variable in four categories. Category four indicates a humid, rank soil with ideal conditions for transmission and hence the maximum soil condition risk is attributed to this category. Category three represents soil with much vegetation and bears an estimated relative contribution of 75 % compared to the maximum soil condition risk since transmission risk in such areas is still high. Category two stands for dry soil with few vegetation which will delay or even reduce the transmission. The relative risk compared to the maximum soil condition risk is set to 25 %. Category one represents stony regions without or with negligable vegetation where no transmission of *F. hepatica* will take place and hence for this category, the soil condition risk is set to 0.

Environmental risk factor forest

Low level solar radiation has a negative effect on the occurrence of *L. truncatula*, as there is an insufficient growth of algae to feed snails (Petzold, 1989). For this reason it is assumed, that there is no risk for fasciolosis in forest. Hence the risk as function of the factor forest can be considered as a binary variable, taking value 0 when forest is present and hence no potential transmission is assumed, else 1 (100%).

Environmental data

The mathematical model used in this paper requires real data of the environmental factors in order to evaluate the overall risk density distribution for each cell of 100 x 100 metres in Switzerland over the year (best resolution possible based on the available data). The mean monthly temperature and rainfall from 1994 to 2004 was obtained from MeteoSwiss (www.meteoschweiz.ch) and from the Atlas of Switzerland (www.atlasofswitzerland.ch), the soil conditions including ground water from the Atlas of Switzerland and the forest distribution in Switzerland also from the Atlas of Switzerland.
The temperature data from 24 representative Swiss meteorological stations were calculated for an altitude of zero meters using regression analysis and the digital elevation model of Switzerland DHM25 (swisstopo). A polynomial interpolation was used to calculate the monthly temperature across the entire area of Switzerland. Afterwards, the temperature was adjusted to altitude also with the digital elevation model of Switzerland DHM25 (swisstopo).

Combination of the enviromental risk factors

To obtain an overall risk density distribution based on the environmental factors as described above, a risk density distribution for each of the risk factors separately is defined. The focused region (Switzerland) was divided into cells. For each cell, empirical data about the four environmental risk factors were available. Where more than one measurement is available for a factor, the average value is taken. Given such a (averaged) measurement *x* for a particular risk factor and given the corresponding risk density distribution *f(.)*, the contributed risk density is simply defined as *f(x).* Since the risk factors are considered to be independent, the overall risk for that cell can be calculated by multiplying the contributed risk densities. This procedure can be applied for each cell at any point in time to obtain finally an overall risk density distribution for the focused region over the whole year based on the environmental factors temperature, rainfall, soil condition and forest.

To obtain the risk density curve based on the risk factor temperature, the following stages are considered: The eggs, *L. truncatula* and the metacercaria. Since the number of cercariae migrating from an infected snail does not correlate with the number of miracidia infecting the snail (Pesigan et al., 1958), a random transmission dynamics between the two stages is assumed. As described, a discrete set of points is available for each stage (egg, snail and metacercaria) indicating the risk at different temperatures derived from the literature. This set is transformed into a continuous density distribution by a filtered polynomial density estimation (Heinzmann, 2007). This density estimation approach provides an algebraic expression for the resulting continuous density curve which simplifies the multiplication of density curves. The risk density distributions of the individual stages can now be combined to the process specific risk density distribution. Multiplying the resulting distributions yields a risk density curve for the environmental factor temperature.

The discrete set of points describing the risk of infection in function of the factor rainfall is transformed into a continuous density distribution using the same filtered polynomial density estimation approach as described above. The resulting continuous risk density distribution for the factor rainfall indicates the risk of infection depending on the amount of rainfall over a certain time period.

Since the two environmental risk factors soil condition and forest are categorical variables, their underlying risk density distributions are discrete and straightforward to implement based on the individual information for soil condition and forest described.

L. truncatula has been found in regions as high as 2100 m in Switzerland (Eckert et al., 1975) and has been recorded at altitudes of 2600 m elsewhere in Europe (Brohmer et al., 1956). However, in alpine regions it takes the parasite two years to complete a life cycle (Eckert et al., 2005).

In addition to the temperature dependent risk, it is also assumed that the risk exponentially decreases in function of height after approximately 1800 m, at 2000 m only 50 % of the relative risk is present and at 2600 m the risk is reduced to 1 % (negligible). The implementation of those assumptions in our model is done as follows: We fit an exponential function of the form $y = a\exp(bx)$, where x is the altitude and y is the proportional presence of the relative risk (e.g. 0.5 for 2000 m). For reasons of simplicity, we subdivide the height into intervals as [0,1800], (1800,2000], (2200,2600] and (2600+). Then for the centres of the intervals, we interpolate from the fitted exponential function the corresponding *y* value which yields the values 0.68 and 0.06 for the centres 1900 m and 2300 m. Finally, the height-dependent threshold is implemented such that the computed risk for altitudes until 1800 m are multiplied by 1, for altitudes in (1800,2000] with 0.68, for altitudes in (2000,2600] with 0.06 and for altitudes larger than 2600, the computed risk is multiplied by 0 meaning that there is no potential risk at such heights.

Map design and visualization technique

The overall risk density for each cell is visualized by a colour scale ranging from red to white representing five risk classes (very high risk, high risk, moderate risk, low risk, very low risk).

In addition, the risk map includes some extra information such as borderlines of cantons, water bodies, relief, capitals of cantons and spatial distribution of the forest in

Switzerland. A special feature is provided by the animation of the monthly risk over the course of the year.

The map is composed of following areas: title and logo, reference map and elements of interaction, layers, legends and the main map (fig. 1). In the logo area the title can be seen. The reference map shows the cut out indicated in the main map. Beneath, the geographic coordinates show the exact position of the mouse pointer. It is possible to scroll on the whole map, to select a new map centre or to zoom into the map. The navigation tools are organised next to the reference map and are labelled with corresponding symbols. The elements of the map are grouped in layers which can be activated or deactivated. A month can be chosen from the selection list. Furthermore, an animation of the monthly risk over the course of the year can be displayed. As the animation runs, the name of the active month is shown. Names of cities and water bodies under the mouse pointer are shown in the info panel. Furthermore, this area shows the status of the map and project information. The legend illustrates the colour scale of the risk. The main map displays the chosen area with the activated elements (fig. 2).

Figure 1: The interactive map for the assessment of mean annual risk for survival, reproduction and transmission of *Fasciola hepatica* and *Lymnaea truncatula* in the environment in Switzerland and its functional areas: Title and logo (1), reverence map and navigation tools (2), layers (3), legend (4), and main map (5).

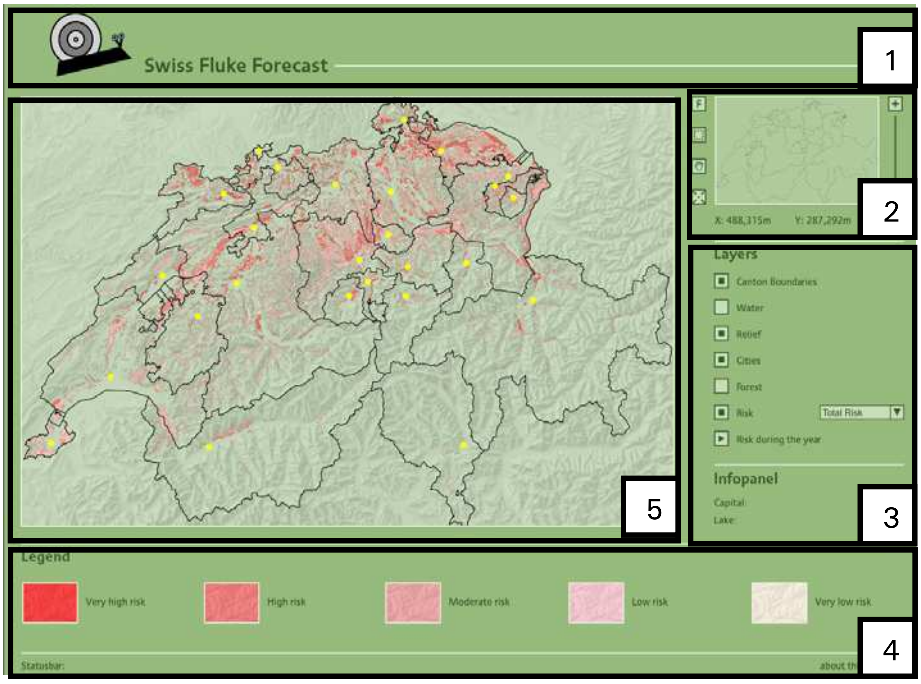
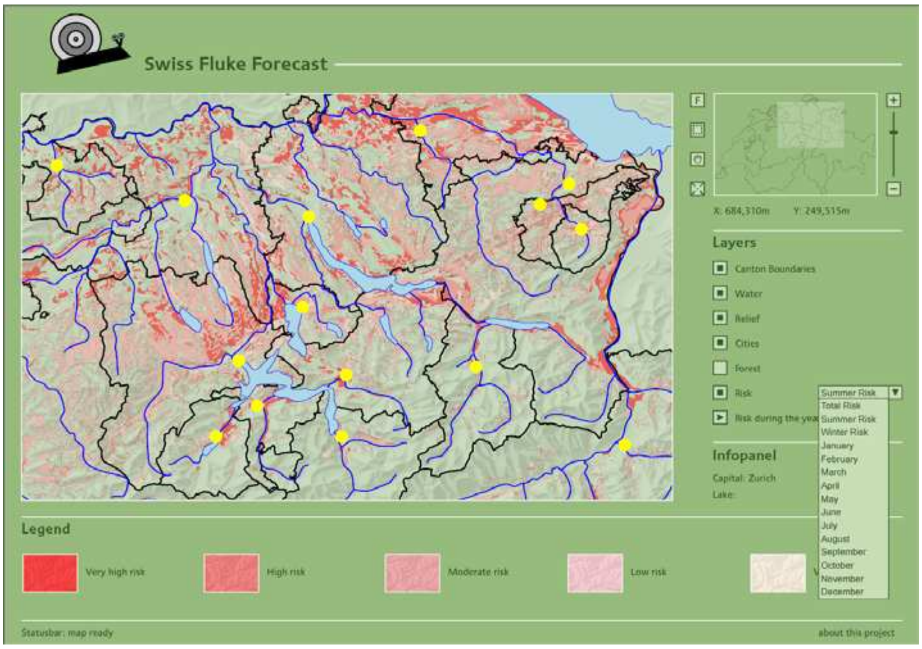


Figure 2: A screenshot of the map showing a section at a larger scale and the opened selection list. The summer risk is displayed with several layers activated.



Results

Risk model

The risk density distributions for the factor temperature for eggs of *F. hepatica*, for *L. truncatula* and for metacercaria as well as the overall temperature risk curve are displayed in figure 3.

The x-axis displays the temperature in °C and the y-axis shows the corresponding density curve which when integrated over the whole temperature spectrum equals one. Hence the whole risk based on the factor temperature is set to one and each density of a temperature range refers relatively to the whole temperature risk.

The risk density distribution for the environmental factor rainfall is displayed in figure 4.

Map

Visualizing the results of the risk model results in island maps without artificial control points. Some typical topographic elements such as lakes and the Alps can be identified (fig. 5). The different risk regions are small spaced and seem to string together arbitrarily.
In August environmental conditions are especially suited for the development, reproduction, and transmission respectively, for *L. truncatula* and the free living stages of *F. hepatica* (fig. 5). Note that the risk values of the other months are graduated according to the risk in August.

Combining the risk maps with the other layers results in the definite map. Figure 1 shows the annual risk for suitable environmental conditions for *L. truncatula* and the free living stages of *F. hepatica* to develop in Switzerland. North of the Alps there is a far broader spread of regions with suitable environmental conditions. Regions in the north-east of Switzerland bear particular hazard of transmission of this parasite. When looking at mountainous regions, hazardous sites can be seen mainly in valleys, often adjacent to bodies of water.

The entire map can be inspected under http://www.carto.net/rapsch/riskmap/.

Figure 3: Relative risk frequency for development and survival of eggs and metacercaria of *Fasciola hepatica* and *Lymnaea truncatula* as a function of temperature as well as the combined relative risk frequency as a function of temperature.

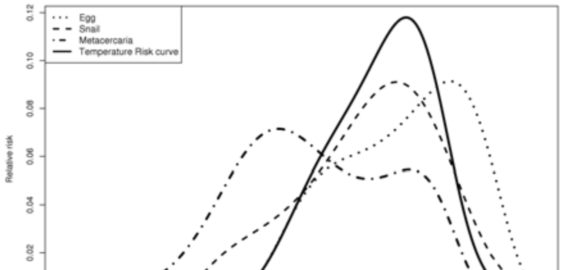


Figure 4: Risk frequency of the parasite cycle as a function of rainfall.

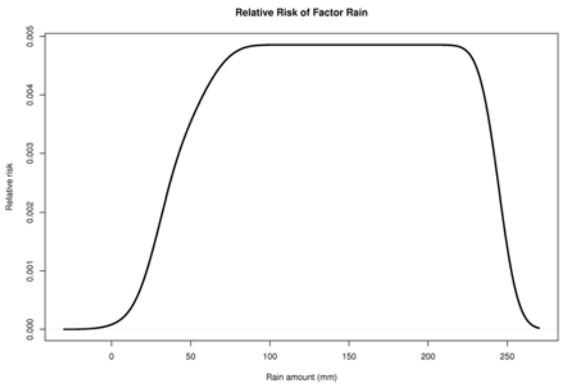
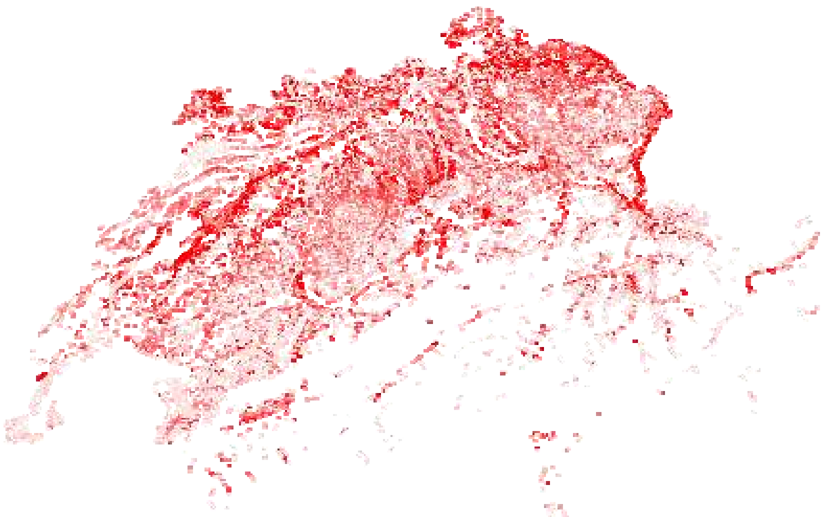


Figure 5: Risk in August.



Discussion

Maps predicting fasciolosis risk have been created for several endemic areas. In Cambodia Tum et al. (2004) created a map based on inundation, proximity to rivers, land use, slope, elevation, and the density of cattle and buffaloes. Other studies in east Africa were based on moisture and temperature (Malone et al., 1998; Yilma and Malone, 1998). However, in none of theses studies, temperature setting of the free-living parasite stages and of the intermediate host have been modelled this detailed as in the study at hand. But in Switzerland, the temperature dependence needs to be taken into account. Even though the data for the model derives from the literature, there remain uncertainties. Missing data was completed by the authors' own observations and from personal communications. Incomplete published data was augmented by interpolation to obtain intermediate data points for a considered range.

Nevertheless, this interactive map at hand is an aiding tool that illustrates the risk for free-living stages of *F. hepatica* and the intermediate host *L. truncatula* to occur subject to suitable environmental conditions.

The model is based on mean monthly temperatures without considering previous months, although the development of the cycle takes more than one month. This may result in an estimation bias of the true risk in some areas especially in high regions, where warm periods are short and the parasite cycle cannot be completed within a year. But since our model completely depends on environmental factors, the estimation of the risk in a cell at a certain time depends on the prevailing conditions in that cell at that time. The integration of a cumulative risk estimation over time would require to model the parasite cycle itself in a temporal and spatial framework, which is out of scope of the present paper.

The existence of permanent snail habitats depends on the geological formations and the topography of a land. These factors are constant and will determine whether or not snail habitats can occur in a given area (Ollerenshaw and Smith, 1969). Rainfall can influence the size of a permanent habitat or the migration distance of snails, but not the occurrence of the intermediate host itself. For this reason, and because the water content of a habitat is dependent on soil type (Wilson et al., 1982), it is assumed, that the occurrence of habitats is mainly detected by the data on soil condition and ground water. For this study, the soils were classified on the basis of water permeability and water logging.

The rainfall model is thought to be sufficient for this study to model monthly variations of the dimension of potential risk regions, as it was not the gain of this study to make an annual forecast for a specific year, but to model regions in Switzerland where *L. truncatula* and the free living stages of *F. hepatica* potentially develop. Furthermore, rainfall is not assumed as a restricting factor in Belgium (Bossaert et al., 1999). This is assumed to be true also in Switzerland.

On the basis of the Swiss elevation altitude model DHM25 a 25 x 25 meters grid could be created with the temperature data. Limiting factors though are the data on rainfall and soil condition (Atlas of Switzerland) with an original scale of 1:200 000. Thus, on the basis of the available data the best resolution was realised. Due to the 100 x 100 meters grid, the presented map comes with a high resolution, and a user can seek potential habitats approaching farm level.

Today, multimedia cartography is used as a modern branch of classic cartography. With this technique, data can be visualized on a computer and provided online. The data can be modified and updated at any time and the target group has access whenever necessary.

When looking at the regional distribution of the risk in Switzerland, there is a remarkable split in two parts through the Alps. North of the Alps there is significantly higher risk of fasciolosis than south of the Alps. As the climate is warmer and dryer south of the Alps than north of them, environmental conditions are suboptimal for the free-living stages of *F. hepatica* and *L. truncatula* in the south of Switzerland. North of the Alps the climate is moderate which makes environmental conditions in some regions better for survival of parasite and intermediate host. Regions with high risk lie in the north-east of Switzerland. Also, in regions around lakes, the risk is found to be high. This is because lakeside ground water levels are relatively high and so the soil contains a lot of humidity (Bitterli et al., 2004) which provides ideal conditions for the free living stages of the parasite and the intermediate host.

In the model, the best environmental conditions for the development of *L. truncatula* and the free living stages of *F. hepatica* in Switzerland are in August. As a result of this, under adequate environmental conditions, high cercarial shedding would occur from late October to November. This finding is consistent with the highest infection risk in late summer and autumn in Europe (Ross et al., 1968; Ross 1970a; Ross 1977; Eckert et al., 2005).

No underlying transmission model of the complete parasite cycle is used to construct the map, thus cumulative effects were not considered. Nevertheless, the map gives a detailed review on regions in Switzerland potentially dangerous to hosts of *F. hepatica*. In a next step, the biology of the hosts, the reproduction, survival time and death rates of each parasite stage as well as of the intermediate host should be integrated in the model.

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3. UNTERSUCHUNGEN ZUR BEKÄMPFUNG DER BOVINEN FASCIOLOSE IN DER SCHWEIZ

3.1. Das Problembewusstsein von Landwirten in bezug auf die Fasciolose des Rindes

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Zusammenfassung

In 54 Schweizer Milchwirtschaftsbetrieben mit dem Bestandesproblem Fasciolose wurden die Landwirte zum Betrieb, zur Ausbildung und zum Wissen über *Fasciola hepatica* befragt. Ziel der Befragung war die Beurteilung des Problembewusstseins der Landwirte bezüglich dieser wirtschaftlich bedeutenden Parasitose. Die Auswertung der Fragebogen ergab, dass 72.2 % der Landwirte von der Fasciolose bei ihren Tieren nichts geahnt hatten. Das Wissen von 51 der 54 (94.4 %) Befragten wurde als gering bis mittelmässig eingestuft, nur vier Betriebsleitern (7.4 %) waren neben dem Zyklus auch die Bekämpfungsmöglichkeiten bekannt. Zusammenhänge zwischen dem Wissen über Fasciolose und den Betriebsdaten oder dem Abschlussjahr der landwirtschaftlichen Ausbildung bestanden nicht. Im Weiteren wurden die erhobenen Daten mit Antworten von 33 nicht betroffenen Landwirten verglichen. Dabei zeigte sich, dass von den Befragten dieser Gruppe 12.1 % der Meinung waren, ihre Tiere litten an Fasciolose.

Das Problembewusstsein von Landwirten in bezug auf die Fasciolose des Rindes

Summary

The owners of 54 cattle herds with bovine fasciolosis, as a livestock problem in Switzerland, were surveyed on their farm, on their education and knowledge on Fasciola hepatica. The aim of the survey was to assess the awareness of the farmers concerning this economically important parasitosis. The analysis of the questionnaires revealed that 72.2 % of the farmers were unaware of the fasciolosis in their animals. The knowledge of 51 out of 54 (94.4 %) respondents was classified as low to moderate, only four farmers (7.4 %) had knowledge of control strategies in addition to the parasite cycle. The knowledge on fasciolosis did not correlate with farm data or year of graduation from school for agriculture. Furthermore, the results were compared to the answers of 33 farmers whose animals were not infected with Fasciola hepatica. Of this group of respondents 12.1 % believed that their cattle suffered from fasciolosis.

Einleitung

Die Fasciolose, ihre wirtschaftliche Bedeutung, sowie Massnahmen zu ihrer Bekämpfung, sind schon lange Zeit Gegenstand intensiver Forschung (Boray, 1972, Ribbeck und Witzel, 1979, Hope Cawdery, 1984). Die wirtschaftlichen Verluste entstehen vorwiegend durch reduzierte Milch- und Mastleistung, reduzierte Fruchtbarkeit und die Leberkonfiskate (Hope Cawdery et al., 1977, Oakley et al., 1979, Randell und Bradley, 1980, Genicot et al., 1991). Bei der Milchleistung entstehen Einbussen bis 15.4 %, bei der Mastleistung bis 28.0 %. Die geschätzten medianen Schäden in der Schweiz belaufen sich auf CHF 81'715'988 (95 %-Konfidenzintervall: CHF 20'608'772 – 207'887'386; Schweizer et al., 2005).

Die Bekämpfung besteht neben der Therapie mittels Triclabendazol (Endex®, Novartis) in der Prophylaxe in Form von Weidemanagement (Boray, 1971, Boray, 1972, Torgerson und Claxton, 1999). Für eine deutliche Reduktion der Prävalenz in einer Herde werden Triclabendazol-Behandlungen alle 12 – 13 Wochen empfohlen (Torgerson und Claxton, 1999). Obwohl Triclabendazol eine sehr gute Wirksamkeit auch gegen juvenile Stadien aufweist, ist es wegen der langen Absetzfrist auf Milch für den Einsatz bei laktierenden Kühen wirtschaftlich unattraktiv (Rapic et al., 1988, Shi et al., 1989; Lebensmittelverordnung vom 1. März 1995). Es ist deshalb sinnvoll, die Infektion nicht nur zu behandeln, sondern die Ansteckung mittels betrieblicher Massnahmen, wie dem Drainieren von Weiden, dem Auszäunen von nassen Stellen auf den Weiden oder mittels Weidewechselsystemen, zu verhindern (Boray, 1972). Bekämpfungsmassnahmen kommen jedoch nur dort gezielt zum Einsatz, wo betroffene Landwirte etwas über Fasciolose wissen und sich des Problems bewusst sind.

Um herauszufinden, wie umfangreich das Wissen von Schweizer Landwirten in bezug auf diese wichtige Parasitose ist, wurden 87 Betriebsleiter zum Thema befragt und ihre Betriebe wurden auf das Vorhandensein des Parasitenzyklus untersucht. Daraufhin wurden die Antworten auf Fragen zur Fasciolose beim Rind allgemein und auf die Sensibilisierung in Bezug auf die Parasitose als Bestandesproblem von 33 nicht betroffenen Landwirten mit denjenigen von 54 betroffenen Landwirte verglichen. Das Ziel war es, mögliche Unterschiede zwischen den beiden Gruppen im Umfang des Wissens und in bezug auf das Problembewusstsein zu finden. Im Weiteren wurde das Wissen der 54 betroffenen Landwirte mit Herdengrösse, Betriebsfläche, durchschnittlicher Milchleistung und Landwirtschaftszone in Zusammenhang gesetzt. Dabei ging es darum, herauszufinden, ob es Unterschiede gibt im Wissensumfang von Leitern von grösseren oder leistungsstärkeren Betrieben verglichen mit Leitern von kleineren oder leistungsschwächeren Betrieben.

Material und Methoden

Von Februar 1999 bis Februar 2000 wurden an einem Ostschweizer Schlachthof alle mit grossen Leberegeln befallenen Tiere identifiziert, die Herkunft mit Hilfe der

Verkehrsscheine bzw. der Begleitdokumente eruiert und die Besitzer kontaktiert. 274 von 3267 (8.4 %) untersuchten Lebern waren befallen (Schweizer et al., 2003). Von den Besitzern dieser 274 Tiere waren 68 an einer weiteren Abklärung auf ihrem Betrieb interessiert. Hinzu kamen Besitzer von Patienten der Klinik für Wiederkäuer des Departements für Nutztiere der Universität Zürich. Bei deren Tieren war an der Klinik ein Befall mit Fasciola hepatica entweder mittels Koproskopie oder Gallenuntersuchung nachgewiesen worden. Insgesamt wurden seit August 1999 aus den Kantonen Aargau, Appenzell Ausserrhoden, Glarus, Luzern, Obwalden, St. Gallen, Schaffhausen, Schwyz, Thurgau, Zug und Zürich 90 Betriebe auf das Vorkommen von Fasciola hepatica untersucht. In die vorliegende Untersuchung wurden nur die Milchwirtschaftsbetriebe eingeschlossen (n = 87).

Der Parasitenzyklus konnte auf 54 Betrieben anhand von parasitologischen Kotuntersuchungen von je fünf Kühen, dem Auffinden des Zwischenwirtes Lymnaea truncatula und dem Nachweis von Fasciola hepatica-DNA mittels TaqMan PCR im Zwischenwirt bewiesen werden. Auf 33 Betrieben waren der Ei-Nachweis im Kot von fünf Tieren negativ und Lebensräume für die Zwergschlammschnecke fehlten. Diese Betriebe dienten als Kontrollgruppe.

Alle Landwirte wurden von derselben Person anhand eines Fragebogens unter anderem zum Betrieb, zum Werdegang und zum Wissen über Fasciola hepatica im Rahmen des Betriebsbesuches mündlich befragt.

Die Fragebögen derjenigen Betriebsleiter, die nachgewiesenermassen Fasciolose im Bestand hatten, wurden in bezug auf folgende Fragestellungen ausgewertet:

1. Wissen die Betriebsleiter, dass Fasciolose in ihrem Bestand ein Problem darstellt?
2. Werden auf dem Betrieb Massnahmen gegen Fasciolose ergriffen?
3. Gibt es für den Betriebsleiter offensichtliche Bestandesprobleme?
4. Werden die Lebern von Schlachttieren konfisziert?
5. Was weiss der Betriebsleiter über Fasciolose?
6. Wann hat der Betriebsleiter die landwirtschaftliche Schule besucht?
7. Besteht zwischen dem Abschlussjahr der Ausbildung und dem Wissen über Fasciolose ein Zusammenhang?

Die 33 Landwirte, die nachgewiesenermassen keine Fasciolose im Bestand hatten, mussten ebenfalls alle Fragen beantworten. Für die vorliegende Untersuchung wurden jedoch nur die Antworten auf die Fragen 1, 3 und 4 in die Auswertung einbezogen und mit den Antworten der 54 Landwirte mit Fasciolose im Bestand auf diese drei Fragen verglichen.

Die statistischen Berechnungen der Mittelwerte, Standardabweichungen, Häufigkeitsverteilungen und der multivariaten Varianzanalysen (ANOVA) erfolgten mit Hilfe des Programms SPSS 11.5.0 (LEAD Technologies Inc.).

Ergebnisse

Betriebe ohne Fasciolose

Die durchschnittliche Herdengrösse betrug 22.2 ± 9.2 Kühe (Mittelwert ± Standardabweichung) und 10.4 (Mittelwert; Min. 0, Max. 42) Jungtiere. Die Kühe wiesen im Durchschnitt eine Milchleistung von 6470 ± 968 kg / Jahr auf und die durchschnittliche Betriebsfläche betrug 22.3 ± 11.7 ha. Es waren Bestände aus Ackerbau-, erweiterter Übergangs-, Übergangs- und Hügelzone vertreten.

Vier Landwirte (12.1 %) nahmen irrtümlicherweise an, dass ihre Tiere Fasciolose hätten. 14 Landwirte berichteten über Bestandesprobleme wie Fruchtbarkeitsstörungen, Mastitiden, Klauenprobleme, peripartales Festliegen und Folgen von BVDV-Infektionen.

17 Betriebsleiter gaben an, dass die Lebern von Schlachttieren häufig oder meistens konfisziert werden.

Betriebe mit Fasciolose

Die durchschnittliche Herdengrösse betrug 21.2 ± 8.4 Kühe und 14.2 ± 12.2 Jungtiere. Die Kühe wiesen eine durchschnittliche Jahresmilchleistung von 6382 ± 727 kg auf und die durchschnittliche Betriebsfläche betrug 20.7 ± 8.3 ha. Es waren Bestände aus allen Landwirtschaftszonen ausser der Bergzone III vertreten.

15 Landwirten (27.8 %) war bekannt, dass Fasciolose in ihrem Betrieb vorkommt, 39 Landwirte (72.2 %) ahnten nichts von der Parasitose. Es bestand kein Unterschied im Problembewusstsein bei den Landwirten mit Fasciolose im Bestand verglichen mit dem Problembewusstsein derjenigen ohne Fasciolose im Bestand ($P > 0.05$, Chi^2-Test).

Fünf von 15 Landwirten, denen der Befall bekannt war, hatten bereits Massnahmen in Form von Drainagen (1 Landwirt) bzw. Behandlung der Galtkühe mit Triclabendazol (4 Landwirte) ergriffen.

Insgesamt berichteten 20 Landwirte über Bestandesprobleme, davon 12 über eine schlechte Fruchtbarkeit bei den Kühen. Im Weiteren wurden das vermehrte Auftreten von Mastitiden, Klauenproblemen, Kälberdurchfällen, reduzierter Milchleistung, Ketose und Abmagerung von den Betriebsleitern beschrieben.

Zwischen den Beständen ohne und denjenigen mit Fasciolose bestand kein Unterschied in der Häufigkeit des Auftretens von Bestandesproblemen im allgemeinen ($P > 0.05$, Chi^2-Test) und von Fruchtbarkeitsproblemen im besonderen ($P > 0.05$, Chi^2-Test).

31 Betriebsleiter gaben an, dass die Lebern von Schlachttieren häufig oder meistens konfisziert werden. Es bestand kein Unterschied zwischen den beiden Gruppen in der Frequenz der Konfiszierung von Lebern bei der Schlachtung ($P > 0.05$, Chi^2-Test).

In Bezug auf das Wissen über Fasciolose wurde dieses bei 18 Landwirten, da der Zyklus nicht bekannt war, als gering beurteilt. 33 Landwirte kannten den Zyklus, wussten jedoch nichts über Bekämpfungsmassnahmen. Ihr Wissen wurde als mittelmässig eingestuft. Vier Landwirten waren sowohl der Zyklus als auch die Bekämpfung des grossen Leberegels bekannt. Ihr Wissen wurde als umfangreich bewertet.

Die landwirtschaftliche Schule war von 42 Betriebsleitern besucht worden, ein Betriebsleiter hatte eine Ausbildung als Metzger. Zwei Landwirte schlossen die landwirtschaftliche Schule 1958 ab, sieben Landwirte schlossen die Ausbildung zwischen 1960 und 1969 ab, 12 zwischen 1970 und 1979, 15 zwischen 1980 und 1989 und sechs zwischen 1990 und 1998. Es konnte kein Zusammenhang zwischen dem Abschlussjahr der Ausbildung und dem aktuellen Wissen über Fasciolose festgestellt werden ($P > 0.05$, ANOVA).

Diejenigen Landwirte, denen bekannt war, dass ihre Kühe mit dem grossen Leberegel befallen sind, wussten durchschnittlich gleichviel über Fasciolose wie die anderen Landwirte, die von der Parasitose nichts gewusst hatten ($P > 0.05$, Chi^2-Test).

Zwischen dem Wissen der Betriebsleiter über Fasciolose und der Betriebsfläche ($P > 0.05$, ANOVA), der Herdengrösse ($P > 0.05$, ANOVA), der durchschnittlichen Milchleistung ($P > 0.05$, ANOVA) oder der Landwirtschaftszone ($P > 0.05$, Chi^2-Test) bestand kein Zusammenhang.

Diskussion

Obwohl mit der Einführung des Triclabendazols in den 80er Jahren die Therapiemöglichkeit der Fasciolose beim Rind viel besser geworden ist, ist die Prävalenz dennoch nicht zurückgegangen (Schweizer et al., 2003). Es wird vermutet, dass der Grund dafür vorwiegend in einem mangelnden Problembewusstsein betroffener Landwirte gegenüber dieser wirtschaftlich bedeutenden Parasitose liegt.

Die Ergebnisse der vorliegenden Arbeit zeigen deutlich, dass die Mehrheit der betroffenen Landwirte nicht wusste, dass ihre Kühe mit Fasciola hepatica befallen waren. Nur etwa ein Viertel der Befragten wusste tatsächlich von dem Befall. Es bestand jedoch kein signifikanter Unterschied zum Anteil der nicht betroffenen Betriebsleiter, die der Ansicht waren, ihre Tiere litten an Fasciolose. D.h. der Verdacht des Befalls mit Fasciola hepatica beruht nicht auf einer tatsächlichen Diagnose sondern basiert hauptsächlich auf dem, in beiden Gruppen auftretenden, häufigen Konfiszieren der Lebern bei Schlachttieren. Werden von Schlachtrindern eines Betriebes die Lebern regelmässig verworfen – die genauen Gründe sind den Landwirten meist nicht bekannt – wird Fasciolose oft als Ursache angenommen. Neben dem Befall mit Fasciola hepatica kann jedoch auch der viel häufigere Befall mit Dicrocoelium dendriticum zum Verwerfen der Leber bei der Schlachtung führen, ebenso wie Veränderungen der Farbe oder der Konsistenz des Organs.

Die meist aus Experimenten bekannten Auswirkungen auf Fruchtbarkeit und Milchleistung (Hope Cawdery et al., 1977, Oakley et al., 1979, Randell und Bradley, 1980, Gründer, 2002), wurden ebenfalls von beiden Gruppen gleich häufig beschrieben. Da bei dieser Befragung das subjektive Empfinden der Landwirte bezüglich der Tiergesundheit und Fruchtbarkeit erfasst wurde, müssen diese Ergebnisse jedoch kritisch betrachtet werden. In Bezug auf den objektivsten Parameter, die Milchleistung, konnte allerdings ebenfalls kein signifikanter Unterschied zwischen den beiden Gruppen festgestellt werden. Es ist bekannt, dass befallene Tiere mehr Futter aufnehmen als nicht befallene (Oakley, 1979), was unter natürlichen Bedingungen eine Leistungseinbusse infolge der Parasitose maskieren kann. Ein Mehreinsatz an Futter bei befallenen Tieren zur Erzielung des gleichen Ertrags wie bei nicht befallenen Tieren ist allerdings ebenfalls ein nachteiliger wirtschaftlicher Faktor.

Der Umfang des Wissens der meisten Betriebsleiter betreffend den grossen Leberegel wurde zum Zeitpunkt der Befragung als gering bis mittelmässig beurteilt. Selbst Landwirte, denen das Problem auf ihrem Betrieb bekannt war, wussten nicht mehr über den Parasiten als Betriebsleiter, die nichts von der Erkrankung geahnt hatten. Ebenso wenig bestand ein Unterschied im Wissen der Leiter von grösseren oder leistungsstärkeren Betrieben und demjenigen von Leitern kleinerer Betriebe.

Die Tatsache, dass zwischen 1992 und 1997 durchschnittlich nur 1.7 % der Rinder in der Schweiz mit Triclabendazol behandelt wurden (Novartis, persönliche Mitteilung) – obwohl die Prävalenz bei dieser Tiergruppe 16.4 % beträgt (Ducommun und Pfister, 1991) – untermauert weiter, dass die Mehrzahl der Landwirte das Problem entweder nicht kennt, oder der Ansicht ist, eine Bekämpfung sei nicht möglich oder nötig.

Wie schon vielfach beschrieben wurde (Hope Cawdery et al., 1977, Oakley et al., 1979, Hope Cawdery, 1984, Genicot et al., 1991, Johnson, 1991), hat diese Parasitose eine grosse tiergesundheitliche und wirtschaftliche Bedeutung. Zwar zeigten sich diese Effekte in der vorliegenden Befragung nicht. Es wurde allerdings auch keiner der erfragten Parameter anhand von Betriebsdaten überprüft. Es ist deshalb dennoch sinnvoll, in verdächtigen Beständen Tiere auf Fasciola hepatica zu untersuchen und gegebenenfalls die Betriebsleiter zu sensibilisieren und zu einer gezielten Bekämpfung zu motivieren. Dem Tierarzt und der Tierärztin kommt in bezug auf ein stärkeres Problembewusstsein der Landwirte gegenüber Fasciolose eine grosse Bedeutung zu.

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3.2. Effizienz der Bekämpfung der bovinen Fasciolose

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Zusammenfassung

Verschiedene Methoden zur Bekämpfung der bovinen Fasciolose wurden beschrieben. Um die Effizienz von Fasciolose Kontrolle zu ermitteln, wurden 32 Milchwirtschaftsbetriebe mit boviner Fasciolose als Bestandesproblem besucht und entsprechend der Lage der Schneckenhabitate in verschiedene Gruppen eingeteilt. 4 bis 5 Jahre später wurden die Betriebe ein zweites Mal besucht und die Betriebe, die die Kontrollvorschläge befolgt hatten, mit denjenigen verglichen, die ungenügende Massnahmen ergriffen hatten.

Die Prävalenz im Endwirt wurde mittels Einachweis im Kot bzw. Antigennachweis im Serum ermittelt.

Die Prävalenz von *F. hepatica* sank signifikant auf den Betrieben, auf welchen die Kontrollstrategien befolgt wurden, verglichen mit den Betrieben, die ungenügende Massnahmen ergriffen hatten.

Efficiency of control of bovine fasciolosis

Abstract

To control bovine fasciolosis, several strategies have been described. To determine the efficiency of an adjusted control strategy, 32 dairy cattle farms with fasciolosis as a herd problem were visited and divided into groups according to the location of the habitats of the intermediate host. Four to five years later, the farms were visited a second time, and the farms which had followed the recommended measures were compared to the ones that did not comply. On both visits faecal samples, on the second visit sera from all cattle were collected in order to determine the prevalence in definite host.

Egg shedding of the definite host was reduced on farms complying with the control recommendations. The seroprevalence in cows on the second visit was significantly lower on farms following the recommendations than on farms not complying.

Introduction

Fasciolosis, caused by *Fasciola hepatica*, is a wide spread parasitic disease in cattle and sheep in Switzerland. Mean prevalence in cattle has been reported to be between 8.4 and 21.4 % (Eckert et al., 1975, Ducommun and Pfister, 1991, Schweizer et al., 2003, Rapsch, 2005, Rapsch et al., 2006). The economic importance and control of fasciolosis have previously been investigated by several authors (Boray, 1972, Ribbeck and Witzel, 1979, Hope Cawdery, 1984). The estimated mean losses in Switzerland add up to EUR 52 millions per year (95 % CI EUR 22 – 92 millions; Schweizer et al., 2005a) resulting in EUR 299 per infected animal. Most of the losses arise from reduced milk yield and reduced fertility, and smaller losses are due to reduced meat production and the confiscation of livers (Schweizer et al., 2005a).

Control measures include anthelmintic prophylaxis such as with triclabendazole (Endex®, Novartis) treatment, pasture management strategies (Boray, 1971, Boray, 1972, Torgerson and Claxton, 1999), and fencing and draining of *L. truncatula* habitats. Even though triclabendazole is highly efficient against adult and juvenile stages of *F. hepatica*, it is rarely used in lactating dairy cows due to the long withdrawal periods on milk (Rapic et al., 1988, Shi et al., 1989). In Switzerland the withdrawal period on milk is 12 days in order to reduce the concentration of triclabendazole to a maximum of 0.01 mg per kg milk (Anonymous, 1995). Therefore cows are often treated with triclabendazole once per year during the dry period, although treatment of up to four times a year may be necessary to attain a significant decrease of the prevalence in a herd (Parr and Gray, 2000, Torgerson and Claxton, 1999).

This study compares the results in terms of reductions of fasciolosis in farms that followed specific control recommendation compared to those farms that were not compliant. In all cases the control strategy was designed according to the specific epidemiological situation on the individual farms.

Material and methods

Between February 1999 and February 2000, 70 herds infected by *F. hepatica* were identified by back tracing animals that had livers confiscated due to fasciolosis at abattoir inspections. 32 of 70 farmers were willing to take part in this study. Their farms were visited in summer 1999 or 2000 and in summer 2004. The autochthonic parasite cycle was demonstrated with coproscopy of five cows per farm (160 cows in total), the finding of *Lymnaea truncatula* and the presence of *F. hepatica* DNA in the intermediate host (quantitative real-time PCR; Schweizer, 2007).

The farms were given control recommendations based on the location of the snail habitats:

- Recommendation A (9 farms): Snail habitats were found on pastures used for young stock or dry cows. Treatment with triclabendazole after bringing the animals off the infectious pasture was recommended.
- Recommendation B (3 farms): Snail habitats were found on all pastures used for dairy cows. It was recommended to treat all animals with triclabendzole staggered

over the winter, preferably during the dry period. Additionally treatment of dry cows during summer period was advised.

- Recommendation C (20 farms): On these farms, habitats were found in single pastures used for dairy cows. Therefore the pasture rotation system described by Boray (1972) was recommended. Animals are turned out on pastures without snail habitats in spring. In June and July cows graze on infectious pastures but must be moved to pastures without snails before shedding eggs (Boray, 1971, 1972). Additionally, cows are treated with triclabendazole once, before grazing on the infectious pastures.

To avoid infection from stable feed, all farmers were instructed to feed the herbage from the infectious acreage either as barn dried hay (with ventilation) or as silage. To prevent introduction of new infections, new animals were treated with triclabendazole before turning them out on pastures with snail habitats.

During the second visit in summer 2004 farms were divided into two compliance groups:

- Group 1 (15 farms): Farmers following the recommendations.
- Group 2 (17 farms): Farmers following the recommendations only partially (i. e. only treating the dry cows, as often recommended by veterinarians) or not at all.

The presence of *F. hepatica* in cattle was examined and the farmers were interviewed in regard to the implementation of the control recommendations.

The control recommendations based on the results of the first sample collection were sent to the farmer and the contract veterinarian in order to enhance compliance. They were also informed about the results of the second collection.

Samples

On both visits, from each farm faecal samples of five cows chosen by the farmers were examined for *F. hepatica* by sedimentation technique (Eckert et al., 2005). Pastures were searched for possible habitats for *L. truncatula*.

On the second visit blood samples of all cattle were examined for antibodies against *F. hepatica* using a commercial test kit (ELISA Fascioliasis Serum and Milk Verification, Institut Pourquier, Montpellier; Reichel, 2002).

Statistics

Office Excel 2002 (Microsoft Corporation, Redmond, WA, USA), SPSS 11.5.0 (LEAD Technologies Inc., Haddonfield, NJ, USA) and the statistical software R 2.2.0 (The R Foundation for Statistical Computing, http://CRAN.R-project.org) were used for the statistical analysis.

The outcome was assessed depending on the compliance to an integral control strategy (group 1) compared to poor control (group 2). A linear modeling approach was used with the proportion of seropositive animals as the dependent variable. This was to ensure that any factors which could independently account for reduction in fasciolosis (other than treatment compliance) were included in the analysis. Independent variables included compliance, number of positive cows on the first visit, month of sampling, altitude of farm, number of stock on the farm, and pasture area. A backward stepwise analysis was undertaken with variables having a $p > 0.25$ being eliminated from the model at each step. The data was also weighted according to the numbers of cattle that were serologically tested on the second visit.

Results

Farms

The herds had a mean size of 21.9 ± 9.5 cows (range 8 – 52. For comparison: Switzerland 2002[1] mean 15.7 cows/owner) and 18.0 ± 15.0 young stock (range 0 – 80). On 25 farms the cows were held in tie stalls and on 7 farms in pens. Mean annual milk yield amounted to 6752 ± 775 kg (Switzerland 2002[1] mean 5500 kg). Mean acreage was 22.5 ± 11.2 ha and mean pasture area 11.8 ± 9.7 ha. Depending on weather conditions, grazing on pastures was permitted for a minimum of 60 days from spring to autumn according to the Swiss animal welfare regulations.

Herbage yield was fed as grass, hay, or silage, depending on weather conditions.

Compliance to the control recommendations

15 farmers reported to have followed the control recommendations and 17 farmers had followed the recommendations partially (mainly treating the dry cows with triclabendazole, even though additional measures were recommended) or not done any control at all (table 1). The 15 farmers applying the recommendations correctly, found them easy to follow. From the 17 farmers not complying, seven farmers could not follow the recommendations for technical reasons: there was either not enough pasture for rotations or the pasture with the snail habitat could not be used for herbage feed, but only for grazing. Three of these seven treated the dry cows with triclabendazole. Despite being informed of the economic importance of this parasitic disease on the first visit, seven farmers did not follow the recommendations due to lack of interest. Nevertheless one of them treated the dry cows with triclabendazole. The management of one farm changed throughout the trial and so the control of fasciolosis was discontinued. One farmer used the wrong anthelminthic (doramectin) and two farmers used the wrong pasture rotation range. They both treated the dry cows with triclabendazole.

[1] www.bauernverband.ch

Table 1: Compliance to the control recommendations on 32 farms with bovine fasciolosis

Affected pastures	Control recommendation	Compliance group					
		1			2		
		Prevalence first visit (faecal egg count)	*Prevalence second visit (faecal egg count)*	*Sero-prevalence second visit*	*Prevalence first visit (faecal egg count)*	*Prevalence second visit (faecal egg count)*	*Sero-prevalence second visit*
Young and / or dry cows	Recommendation A: Triclabendazole after bringing off affected pasture	20.0 % (5 farms)	8.0 % (5 farms)	15.5 % (129 cattle)	25.0 % (4 farms)	45.0 % (4 farms)	51.5 % (101 cattle)
All	Recommendation B: - All animals: Triclabendazole in winter - Cows when dry all year	10.0 % (2 farms)	0 % (2 farms)	8.6 % (58 cattle)	0 % (1 farm)	60.0 % (1 farm)	45.0 % (20 cattle)
Single dairy cows	Recommendation C: - Triclabendazole once before grazing on affected pasture - Pasture rotations according to Boray (1971, 1972)	32.5 % (8 farms)	12.5 % (8 farms)	29.8 % (178 cattle)	40.0 % (12 farms)	28.3 % (12 farms)	68.1 % (238 cattle)
Total		30.7 % (15 farms)	9.3 % (15 farms)	21.4 % (365 cattle)	34.1 % (17 farms)	34.1 % (17 farms)	62.1 % (359 cattle)

Prevalence in cattle

Of the faecal samples tested 32.5 % were positive for *F. hepatica* on the first visit. On the second visit 22.5 % of the samples were positive. On farms with poor control the proportion of positive samples did not change. On the farms following the control recommendations the percentage of positive faecal samples decreased from 30.7 to 9.3 % (P = 0.003, χ^2). On the first visit no difference was found between the three treatment groups (P = 0.64, χ^2), but on the second visit the difference was significant (P = < 0.001, χ^2).

On the farms complying with the recommended control strategies, the mean seroprevalence in cattle was 21.4 %. In contrast on farms with poor compliance the mean seroprevalence was 62.1 % (P <0.001, χ^2).

The results of the multivariate linear regression model verified that the decrease in seroprevalence was associated with good compliance rather than confounded by a correlated variable (P < 0.0001; table 2). Herds with a high prevalence on the first visit also had a high prevalence on the second visit. In addition the number of coprologically positive cattle detected on the first visit was also associated with the level of seropositivity detected on the second visit. All other factors (month of sampling, altitude of farm, stocking density and size of farm) were not significant in the model.

Table 2: Significant parameters in the linear model for prevalence of seropositivity in the study farms

	Parameter value	**Standard error**	**P value**
Intercept	0.12	0.055	0.0328
Poor compliance	0.36	0.066	<0.0001
Number of cattle coprologically positive on first visit	0.07	0.024	0.0044

Discussion

Because bovine fasciolosis is an important parasitic disease in Switzerland and economic losses amount to several thousand euros per affected farm, control of this parasite is not only important for animal welfare but also for economic reasons.

Even though several control strategies have been described (Boray, 1971, Harris and Charleston, 1971, Boray, 1972, Armour, 1975, Schneider et al., 1975, Whitehead, 1976), to the authors' knowledge, no studies have investigated the efficiency of control measures designed according to the individual epidemiological situation on a given farm. As the epidemiological situation varies among the farms, the control recommendations were based on location, utilization and number of infectious pastures. A total of 17 farmers failed to followed the recommendations. The 15 farmers complying with the recommendations found them easy to follow. Despite being informed about fasciolosis and the recommended measures of control, seven farmers were not motivated to take measures against the infection. Seven farmers showed interest in implementation of control, but did could not apply the recommendations because of the location of the grazing land on their farms.

Interviews during the first visit revealed that most of the farmers were not aware of the economic importance of bovine fasciolosis (Schweizer et al., 2005b). Due to the information given on this first visit within the scope of the interview, some 22 farmers were now educated about fasciolosis which shows the importance of appropriate information given by veterinarians to farmers.

Six farmers commenced treating their dry cows with triclabendazole (Endex®, Novartis) after the first visit, but failed to implement further measures for the reasons mentioned above. This single treatment is a method often suggested by veterinarians, as it is easy to accomplish and has the lowest economic losses due to milk withdrawal. The present study shows, however, that a treatment of the dry cows on its own does not decrease the prevalence of *F. hepatica* within the herd. The treatment of an individual animal is reasonable to reduce its parasitic load, especially during the initial phase of lactation, but on the herd level the present data suggests this strategy is ineffective. One treatment will not prevent cattle being reinfected, especially during pasture season. A continuous intervention programme is needed to lower the level of infection in snails which will then lower the infection pressure to cattle (Parr and Gray, 2000).

Wherever possible, the rotational system described by Boray (1971, 1972) is recommended, as it is easy to carry out and no structural measures, such as building drainages, are necessary.

If a rotational system is not applicable, treatment with triclabendazole twice a year is the measure of choice: A treatment in winter will prevent shedding of eggs in spring and result in a delayed conclusion of the parasite cycle. An additional treatment of dry cows preferably in late summer or autumn is beneficial for the cows' health. The successful reduction of parasite prevalence on two farms that followed recommendation 2 may suggest that the key interference with the parasite cycle is provided by the treatment in winter and not by the treatment of dry cows. The importance of the winter treatment has been demonstrated in sheep (Taylor et al., 1994). For the practical implementation of this treatment, small groups of cows can be treated staggered over the winter to minimize the economic losses due to the long withdrawal period of 12 days.

To decide, which integral control strategy will bring the best result on a farm, it is essential, to assess the location(s) of snail habitats on the pastures. Complying with our recommendations appeared to significantly reduce the prevalence of *F. hepatica* in the definite host.

A high prevalence on the first visit resulted in a higher prevalence on the second visit independent of compliance. For farms with high herd prevalence prior to control management this would mean, it takes longer to decrease the prevalence on a herd level. Farmers and veterinarians should be aware of this.

In this study, only farms were considered where animals become infected directly by grazing on pastures. Alternatively, the parasite cycle can be maintained by deploying manure on hayfields and feeding the grass in the stable. If this is the case, the grass should be treated prior to feeding, either by barn dried hay (ventilation) or silage, as metacercariae will not survive these procedures (Enigk and Hildebrandt, 1964, Enigk et al., 1964).

Conflict of interest

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Zusammenfassung und Schlussfolgerungen

Das erste Kapitel der vorliegenden Habilitationsschrift befasst sich mit der Prävalenz von *Fasciola hepatica* in Zwischen- und Endwirt in der Schweiz. Mit dem Ziel, aktuelle Prävalenzdaten beim Endwirt Rind zu erheben, wurden an einem Ostschweizer Schlachthof von Februar 1999 bis Februar 2000 die Lebern von 3267 Schlachtrindern im Rahmen der Fleischuntersuchung auf das Vorkommen von *Fasciola hepatica* und *Dicrocoelium dendriticum* untersucht. In 8.4 % der Lebern konnte der grosse Leberegel nachgewiesen werden. Verglichen mit Studien aus den 70er und 90er Jahren des letzten Jahrhunderts hatte sich die Prävalenz der bovinen Fasciolose in der Schweiz wahrscheinlich nicht verändert.

Anhand der Begleitdokumente konnten die mit *F. hepatica* befallenen Tiere zurückverfolgt werden und ein Teil der Besitzer wurde angefragt, ob sie an einer weiterführenden Abklärung der Fasciolose in ihrem Bestand interessiert wären. Auf 70 Betrieben fand daraufhin ein Betriebsbesuch statt und mit Hilfe der Landwirte wurden verdächtige Stellen auf den Weiden auf das Vorkommen des Zwischenwirts, der Zwergschlammschnecke *Lymnaea truncatula* abgesucht. Bei der Zwergschlammschnecke handelt es sich um eine feuchtigkeitsliebende Sumpfschnecke, die typischerweise Drainagegräben (Abb. 1), Hangwasseraustrittsstellen (Abb. 2), Rietflächen (Abb. 3), verschlammte Weidebrunnen (Abb. 4, 5) und die Ufer langsam fliessender Bäche bewohnt.

Potentielle Lebensräume wurden abgesucht, gefundene Zwergschlammschnecken wurden eingesammelt und die Fundorte auf Karten festgehalten. Insgesamt konnten 4733 Schnecken aus 130 Habitaten (36 Bäche, 21 Brunnen, 24 Drainagegräben, 33 Hangwasseraustrittsstellen, 14 Riete, 1 Drainageschaft und 1 Teich) gesammelt werden. Die Habitate befanden sich auf 71 Milchkuhweiden, 39 Jungtierweiden, 14 Mähwiesen und 6 Galtkuhweiden. Mittels quantitativer real-time PCR liess sich in 331 von 4733 Schnecken (7.0 %) *F. hepatica*-DNS nachweisen. 51 Populationen waren infiziert.

Abbildung 1: Sumpfiger Grund eines Drainagegrabens in einer Kuhweide

Abbildung 2: Hangwasseraustrittsstelle in einer Rinderweide

Abbildung 3: Rietfläche mit einer Kuhweide im Hintergrund

Abbildung 4: Weidebrunnen in einer Rinderweide (a) mit zahlreichen Exemplaren von *Lymnaea truncatula* innen an der Brunnenwand (b)

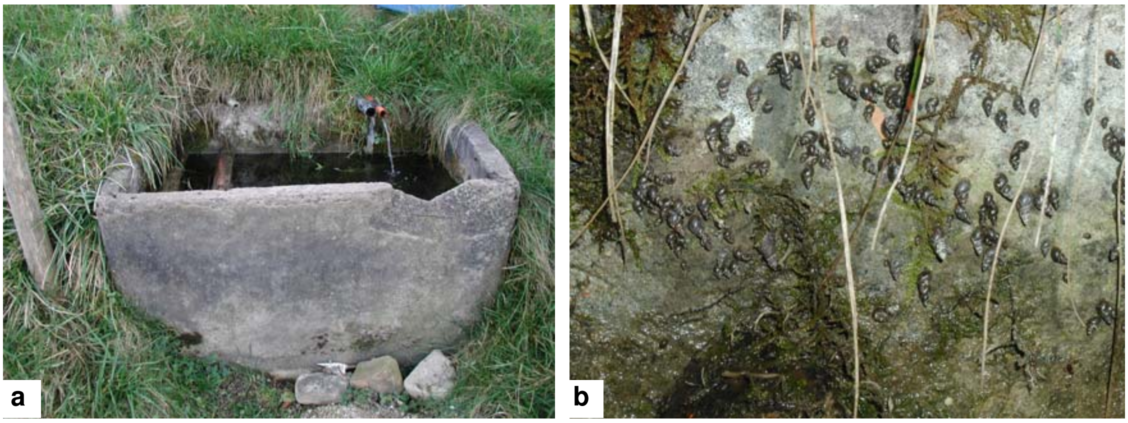

Abbildung 5: Weidebrunnen, welcher *Lymnaea truncatula* als Habitat dient, in einer Kuhweide

Da die makroskopische Beurteilung der Leber im Rahmen der Fleischuntersuchung eine wenig sensitive Methode zum Nachweis eines Leberegelbefalls ist, wurden in einer zweiten Schlachthofstudie 2003 und 2004 Gallenblasen, Blut und Kot von 1331 Rindern an zwei Schlachthöfen untersucht. Die Schätzung der diagnositschen Sensitivität und Spezifität der durchgeführten Untersuchungen sowie der wahren Prävalenz der bovinen Fasciolose fand mittels Bayesischer Techniken (Markov Chain Monte Carlo) statt.

Die wahre Prävalenz von *Fasciola hepatica*-Infektionen wurde auf 18.2 % (95 % Vertrauensintervall 15.0 - 20.0 %) geschätzt. Die Gallenuntersuchung erwies sich als sensitivste Nachweismethode. Die Sensitivität der Koproskopie konnte auf 90 % angehoben werden, wenn Kotproben wiederholt untersucht wurden. Die Fleischuntersuchung wies erwartungsgemäss mit 64 % die schlechteste Sensitivität auf.

Diese Resultate zeigen, dass die Prävalenz der bovinen Fasciolose höher, als bisher aufgrund der Fleischuntersuchung vermutet, ist.

Im zweiten Kapitel wird die Bedeutung der bovinen Fasciolose in der Schweiz auf Basis von zwei Projekten verdeutlicht. Die erste Studie schätzt anhand eines mathematischen Modells die wirtschaftlichen Verluste, die jährlich durch bovine Fasciolose in der Schweiz entstehen. Sogar leichtgradige Infektionen mit dem grossen Leberegel, ohne Anzeichen von klinischen Symptomen, können signifikante Leistungseinbussen bei Rindern bewirken. Deshalb wurden die einzelnen wirtschaftlichen Einbussen, welche durch *Fasciola hepatica* entstehen und in der Literatur beschrieben sind, mittels Tabellenkalkulation aufsummiert und die Variabilität der veröffentlichten Daten mittels Monte-Carlo-Methode modelliert. Das Resultat deutet darauf hin, dass sich die medianen finanziellen Verluste durch bovine Fasciolose in der Schweiz jährlich auf schätzungsweise CHF 88'414'388 (EUR 56'675'890) mit einem 95 % Vertrauensintervall von CHF 14'313'081 - 251'344'655 (EUR 9'175'052) belaufen. Der Hauptteil der Verluste entsteht durch reduzierte Milchleistung und reduzierte Fruchtbarkeit. Verluste durch geringere Fleischproduktion und die Leberkonfiskate machen einen geringeren Anteil am Gesamtverlust aus (Tab. 1).

Anhand der zweiten Studie, einer interaktiven Karte, soll die potentielle Verbreitung des grossen Leberegels in der Schweiz bildlich dargestellt werden. Dafür wurden die für das Überleben und die Fruchtbarkeit der freilebenden Parasitenstadien und des Zwischenwirts geeigneten Umweltbedingungen modelliert. Die Karte basiert auf Temperatur- und Niederschlagsdaten, Bodenbeschaffenheit inklusive Grundwasser und Forstbestand in der Schweiz. Es wurden ausführliche Informationen über die Beeinflussung der Larvenstadien des grossen Leberegels und des Zwischenwirts *L. truncatula* durch diese Umweltbedingungen verwendet, um die interaktive Risikokarte zu erstellen.

Tabelle 1: Die Zusammensetzung der durch *Fasciola hepatica* entstehenden wirtschaftlichen Verluste in der Schweiz

Ursache des Verlustes	**Anteil am Gesamtverlust (in %)**
Reduzierte Milchleistung	67.8
Verlängertes Serviceintervall	25.5
Zusätzliche Besamungen	5.6
Geringere Gewichtszunahme	0.8
Leberkonfiskate	0.2
Behandlung mit Triclabendazol	0.1
Total	100.0

Das dritte Kapitel schliesslich befasst sich mit der Bekämpfung der Parasitose. Da eine Bekämpfung nur dort stattfindet, wo sich die Landwirte des Problems auch bewusst sind, wurden die Leiter von 87 Schweizer Milchviehbetrieben zum Wissen um *F. hepatica* befragt. Auf 54 Betrieben konnte der Zyklus nachgewiesen werden, 33 Betriebe hatten keine Fasciolose im Bestand. 72.2 % der Landwirte, deren Kühe an der Parasitose litten, hatten nichts davon gewusst. Allerdings waren von den nicht betroffenen Landwirten 12.1 % der Meinung, ihre Tiere wären mit dem grossen Leberegel infiziert. Das Wissen um den Zyklus und die Bekämpfung wurde insgesamt als gering bis mittelmässig eingestuft. Aus dieser Studie wird deutlich, dass dem Bestandestierarzt bzw. der Bestandestierärztin in bezug auf die Sensibilisierung der Landwirte eine grosse Bedeutung zukommt.

Die zweite Studie in diesem Kapitel befasst sich schliesslich mit dem Nachweis der Effizienz einer gezielten Behandlung. Dafür wurden an 32 der ursprünglich 70 Betriebe Prophylaxevorschläge abgegeben. Diese basierten auf der Lokalisation der Infektionsquelle. 4 bis 5 Jahre später wurden die Betriebe ein zweites Mal besucht und die Betriebe, die die Kontrollvorschläge befolgt hatten, mit denjenigen verglichen, die ungenügende Massnahmen ergriffen hatten.

Die Prävalenz im Endwirt wurde mittels Einachweis im Kot bzw. Antigennachweis im Serum ermittelt.

Die Prävalenz von *F. hepatica* sank signifikant auf den Betrieben, auf welchen die Kontrollstrategien befolgt wurden, verglichen mit den Betrieben, die ungenügende Massnahmen ergriffen hatten.

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